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A NEW APPARATUS FOR DETERMINING THE RELATIVE VELOCITIES OF IONS; WITH SOME RESULTS FOR SILVER IONS.[1]

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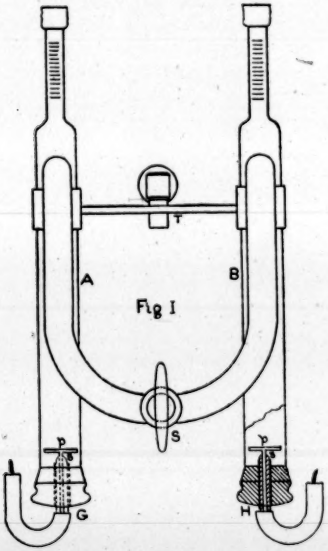
This investigation was undertaken for the purpose of determining the *relative velocities of the ions of a number of silver salts in water, and in methyl and ethyl alcohols.* The work thus far has had to do chiefly with the improvement of the apparatus to be used, the experiments being limited to solutions of silver nitrate in water and in ethyl alcohol, and silver acetate in water.

The Apparatus.

An examination of the results obtained by previous experimenters makes it clear that there must have been serious errors in the methods which they employed. Although all of these methods depend upon the same principle, *i. e.*, the passage of a known amount of current through the solution of the electrolyte, and the subsequent separation and analysis of the solutions around the anode and cathode, yet the velocities as determined differ greatly even in experiments conducted under similar conditions. Previous investigators have relied

[1] From the Dissertation of the author, submitted to the Board of University Studies of the Johns Hopkins University, for the Degree of Doctor of Philosophy. June, 1897.

upon the difference in specific gravity resulting from the electrolysis to secure a separation of the two portions of the solution, the anode and cathode being placed at different levels in the apparatus used. No means were, however, provided for a final division of the liquid at the close of the electrolysis, and the discrepancies in the results are probably to be explained by an incomplete separation of the anode and cathode



portions, either during the experiment or at the close when they are drawn off for the purpose of analysis.

It seemed desirable, therefore, at the start to devise a form of apparatus which should be, as far as possible, free from these defects. The objects in view were: First, that it should be symmetrical in form, permitting the passage of the current

in either direction; second, that it should contain a sufficient quantity of solution to allow a large change in concentration, and thus permit of greater analytical accuracy; third, and most important, that there should be a means of completely separating the solution at the close of the electrolysis, into two portions of accurately determined amount; fourth, that the possibility of diffusion should be, as far as possible, prevented.

A large number of experiments were made with several pieces of apparatus, and the form here described was decided upon as best fulfilling the desired conditions (Fig. I.). It was made of glass, by Emil Greiner of New York.

The two limbs (A and B) are about 18 cm. long, 2 cm. in diameter, and are then contracted above for an additional length of 6 cm. to a diameter of 0.5 cm., the reduced portions being graduated in millimeters. These limbs are connected near the upper ends of the larger portions by a ||-tube about 0.5 cm. in diameter, the bottom of which is some 2 cm. above the level of the lower ends of the limbs. At the center of the tube is a stop-cock (S) of large bore. The lower ends of the limbs are closed by glass stoppers (G and H), through which holes were bored for the insertion of the electrodes. These latter were made of pure silver prepared by reduction of washed silver chloride, which in turn was precipitated from the nitric acid solution of pure mint-silver. Each consists of a round plate (p), autogenically welded to a silver stem (s), which is firmly secured in the glass stopper by means of a piece of pure gum tubing, which was placed in the hole in the stopper and the stem forced through it. To the outer end of the stem was soldered a flexible conductor which was covered by pure rubber tubing to prevent leakage of the electric current, when the apparatus is placed in the thermostat. In order to permit of leveling the apparatus and also of securing it in the bath, the apparatus was cemented into a brass frame, which, by means of a tube and clamp screw can be secured to an upright rod upon the table or in the bath.

For the determination of the effect of temperature, a form

of bath was constructed by the writer which proved extremely efficient, remaining constant to 0°.1 for long periods of time. It consists (Fig. II.) of a double-walled copper vessel with concave

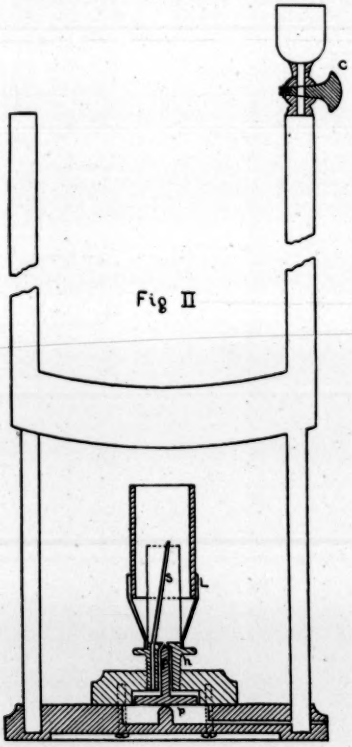


Fig II

bottom, the space between the walls being filled, by means of a stop-cock and cup (C), with a mixture of glycerin and water,

which was employed both because of its large expansion coefficient and because of its preservative action upon the rubber diaphragm. From this annular space communication is made with a space below the diaphragm beneath the bunsen burner (L). Resting upon this diaphragm is a small conical peg (P), which, by motion of the diaphragm through expansion of the liquid, controls the supply of gas which enters through a side tube (not shown) above the diaphragm. A minute constant flame is supplied by a small tube (S) extending from the gas chamber above the diaphragm to the top of the burner tube. The burner itself can be raised from the peg (P) by means of a threaded connection (h) with the gas chamber, and by means of this adjustment and the stop-cock and cup before mentioned, any desired temperature above that of the room can be easily obtained. The arrangements of the diaphragm and burner are due to Professor Henry Carmichael, of Boston. It is, however, perfectly feasible to use the vessel in connection with a thermo-regulator of the type designed by Professor Ostwald, the annular space taking the place of the glass bulb commonly employed. The advantages secured by this arrangement are that the expanding liquid is alone subject to any marked change of temperature, and the water of the bath being thus protected is maintained at a practically constant temperature without recourse to a stirrer.

For determinations at 0° the apparatus was placed in a copper tank filled with finely broken ice, the tank being jacketed with a thick layer of powdered cork and a wooden case.

The electric current, which was supplied by a battery of 40 storage cells, was measured by means of two silver voltameters consisting of platinum crucibles and pure silver anodes, the supports for the crucibles and all electric connections being insulated with vulcanite and mica. The crucibles were heated, cooled in a desiccator, and weighed before each experiment, and the deposit formed by the current was treated in accordance with the directions given by the Committee of the National Academy of Sciences. During the early stages of the investigation the attempt was made to avoid the use of voltameters by employing a form of d'Arsonval galvanometer,

designed by Professor Rowland, the same being calibrated by means of a voltameter. In order to secure constancy, in spite of the varying temperature of the room, all the resistances used in the main circuit and galvanometer shunt were placed in a constant-temperature oil-bath, of the same construction as the one already described. It was found, however, after a number of experiments had been made, that, possibly owing to changes in the suspension filament or to the effects of temperature variations, sufficiently accurate results could not be secured, and further use of the same was abandoned, save as a control, readings being taken every few hours.

Preparation of Solutions.

The analyses were made by titration with ammonium sulphocyanate solutions, ferric ammonium sulphate being used as an indicator. Special care was taken in the preparation of the solutions, and by frequent comparisons with freshly prepared standards of potassium chloride, their strength was accurately determined, the error being not more than 0.02 cc. in 90 cc., or less than 1 part in 4,000. In the preparation of the potassium chloride standards small amounts of the salt were taken, dried in an air-bath in weighing tubes at 140° C., cooled in a desiccator, and weighed. These were then dissolved in water and titrated against silver nitrate. The salt used was purified by repeated crystallization by Dr. H. C. Jones, who also kindly prepared the silver acetate. The ferric ammonium sulphate was made under the direction of Dr. Renouf, while the silver nitrate was in part made from pure silver, prepared as described before, and in part from the commercial article, freedom from acid being secured by repeated recrystallizations. The water used in the preparation of the solutions and for the washing out of the apparatus was obtained by redistillation of ordinary distilled water, to which a solution of chromic acid had been added, the steam passing through a strong boiling alkaline solution of potassium permanganate. The apparatus was arranged according to the plan described by Jones and Mackay.[1] The abso-

[1] Ztschr. phys. Chem., **22**, 237.

lute alcohol was prepared by distillation from lime and subsequent further dehydration with copper sulphate, and the dilute solutions were made from this, their exact strengths being determined with an alcoholometer.

In the preparations of the solutions in absolute alcohol and also in filling the apparatus with the same, special care had to be taken in order to prevent the absorption of moisture, since pure alcohol is an extremely deliquescent substance. The method adopted was as follows: The silver nitrate being placed in a dry liter flask, the latter was filled by means of a siphon from the bottle containing the alcohol dehydrated over copper sulphate, an intermediate filter being introduced to prevent the passage of any particles of the sulphate which the alcohol might contain in suspension. The top of the funnel was closed by a glass cap, the joint between being carefully ground, and the flow of the liquid was started by aspiration through the drying tube, which was connected through a hole in the cap. For this drying tube, as well as those which permitted the entrance of air to the bottle and flask, long U-tubes filled with dehydrated copper sulphate and glass wool were used. The resulting solution of silver nitrate was removed from the flask to a dry bottle in a similar manner, the intermediate filter, however, not being employed. This bottle was permanently closed by a rubber stopper (which had been previously boiled in silver nitrate), connection with the interior being made by a glass tube and drying tube, the openings being capped with rubber tubes and glass plugs. When it was desired to fill the burette for the purpose of analysis or to fill the apparatus, these plugs were removed and the point of the burette inserted into the tube, the rubber tube making a tight joint. The solution was then drawn up into the burette by aspiration through the drying tube, which closed its upper end. In filling the apparatus, the end of the burette was similarly inserted into one limb of the apparatus, the other limb being closed with a drying tube. After filling, both limbs were connected by means of rubber tubes with a series of drying tubes, in order to allow of equality of pressure inside and outside of the apparatus.

The flasks and burettes used were calibrated by means of the apparatus designed by Morse and Blalock,[1] calibration being repeated at least twice.

Method of Analysis.

In the analysis of the solutions after electrolysis it was noticed that if, after the first faint change of color was secured, the solution was allowed to stand for some time, the color grew gradually much darker, and if a known amount of silver nitrate solution of strength equal to the sulphocyanate was then added and the titration again completed, less than the same amount of the sulphocyanate solution was required to cause a return of the color. It was generally necessary to repeat this process several times before a constant state was reached. If, however, when this had been obtained, the solution was allowed to stand, the first tinge of color remained constant for several hours, but finally faded out. The following results of one analysis will render this clear:

$AgNO_3$ N/10. Cubic centimeters.	NH_4CNS N/10. Cubic centimeters.	Corrected amount of NH_4CNS N/10. Cubic centimeters.
0	94.89	94.89
0.23	95.06	94.83
0.51	95.30	94.79
0.74	95.50	94.76
1.17	95.89	94.72
1.83	96.55	94.72
2.18	96.90	94.72

It was noticed that the precipitate first formed was very flocculent, even with the most rapid stirring, but gradually, if the titration were repeated as above, it became granular and settled quickly. We may possibly explain the above results by supposing, as has been found in other cases, that some of the sulphocyanate was occluded in the precipitate first formed.

The sharpness of the color change was increased by the use of a stirrer formed of a bent glass rod which, by means of a

[1] This JOURNAL, 16, 479 (1894).

flexible shaft and suitable connection, was rapidly rotated by an electric motor.

The analysis of the alcoholic solutions at first presented some difficulty, as no data could be found as to the effect of alcohol upon the accuracy of the Volhard method. A series of comparisons was, therefore, made of N/10 solutions of silver nitrate and sulphocyanate, different amounts of alcohol being added. In concentrated alcoholic solutions it was found impossible to secure good results, the nitric acid reacting upon the alcohol and causing the formation of polymeric aldehydes ("aldehyde-gums"), the brownish color of which prevented the determination of the point of color change of the indicator. Success was, however, secured when the alcoholic solution was diluted to about ten times its original volume before addition of the acid, the latter being added slowly with rapid stirring of the solution. The following determinations will render this evident: The figures give in cubic centimeters the amount of silver nitrate taken, the amount of alcohol, water, indicator, and acid added, and the amount of ammonium sulphocyanate (N/10) necessary to cause a faint permanent change in color.

	$AgNO_3$.	C_2H_5OH.	H_2O.	$FeNH_4(SO_4)_2$.	HNO_3.	NH_4CNS N/10.
I.	48.465	50	500	10	20	48.455

Hence 1000 cc. $AgNO_3$ = 999.8 cc. NH_4CNS N/10.

II.	26.365	20	200	10	20	26.355

Hence 1000 cc. $AgNO_3$ = 999.6 cc. NH_4CNS N/10.

The following three comparisons of a solution of silver nitrate in absolute alcohol with the N/10 solution of sulphocyanate, which were made at different times, further illustrate the accuracy of the method:

	$AgNO_3$.	H_2O.	$FeNH_4(SO_4)_2$.	HNO_3	NH_4CNS N/10.
I.	45.520	500	10	15	46.025

Hence 1000 cc. $AgNO_3$ = 1082.3 cc. NH_4CNS N/10.

II.	41.30	500	10	20	44.68

Hence 1000 cc. $AgNO_3$ = 1081.9 cc. NH_4CNS N/10.

III.	42.845	500	10	20	46.330

Hence 1000 cc. $AgNO_3$ = 1081.4 cc. NH_4CNS N/10.

Calibration of Apparatus.

For the purpose of calibration the apparatus was clamped to an upright rod permanently secured to the table, and then filled with mercury, the electrodes being protected by a thin coating of shellac, and all air bubbles carefully removed. The central stop-cock was then turned and the separate portions poured out and weighed, the contents of the stop-cock being included with one-half. In this manner the capacity of each limb was accurately determined. Three separate calibrations were made of each of the two pieces of apparatus employed, at the beginning, middle, and close of the time during which the experiments were made, the results of which are given below. The corrections made are calculated from the losses in silver on the electrodes, due to the action of the current, as given by the deposits in the silver voltameter for the different experiments during the time between the calibration in question and the final one.

Apparatus I.

	Per cent.	Corrections.	Corrected per cent.
Calibration I., Oct. 20, 1896:			
Right side and stop-cock contains	47.381	+0.025	47.406
Left " "	52.619	—0.025	52.594
Calibration II., Dec. 29, 1896:			
Right side and stop-cock contains	47.392	+0.012	47.404
Left " "	52.608	—0.012	52.596
Calibration III., Mar. 30, 1897:			
Right side and stop-cock contains	47.408		47.408
Left " "	52.592		52.592

Apparatus II.

	Per cent.	Corrections.	Corrected per cent.
Calibration I., Oct. 20, 1896:			
Right side and stop-cock contains	51.257	—0.001	51.256
Left " "	48.743	+0.001	48.744
Calibration II., Dec. 29, 1896:			
Right side and stop-cock contains	51.260	—0.006	51.254
Left " "	48.740	+0.006	48.746
Calibration III., Mar. 30, 1897:			
Right side and stop-cock contains	51.253		51.253
Left " "	48.747		48.747

Detailed Description of an Experiment.

The apparatus being fastened to a fixed rod was filled from a burette with the given solution to a mark about half way up the smaller graduated portion of the limbs, and the exact position noted. The reading of the burette and the temperature of the room were also taken. The open ends of the limbs were then closed by stoppers having capillary openings to prevent any increase or decrease of internal pressure due to heating or cooling in the baths, and the whole was secured in the bath (by means of a vertical rod as before) at such a height that the level of the liquid inside and the water outside were practically the same. Connections with the battery and voltameter being made, the electrolysis was allowed to proceed for several hours, the time being regulated according to the strength of the current, as indicated by the galvanometer deflections, these latter also affording a test of the constancy of the current. At the close of the electrolysis a little wax was placed over the capillary openings to prevent any disturbance of the level of the liquid, and the apparatus removed from the bath and clamped to the fixed rod. The wax now being removed, the level of the liquid in the two limbs was brought to equal numbers above or below the fixed marks on the graduated limbs, and the central stop-cock turned. After the whole had acquired the temperature of the room the height of the liquid in the limbs was again noticed, any error due to leakage through the stop-cock or stoppers being thus at once detected. The contents were then carefully washed out, filtered through washed asbestos to remove any disintegrated silver, and analyzed. The amount of silver as found by the analysis was compared with that calculated from the amount of solution placed in the apparatus at the beginning of the experiment, and the accuracy of the result was thus determined. The calculation of the velocity of the ions was then very simple. Thus, in one experiment the apparatus contained at the start 92.965 cc. of N/10 solution of silver nitrate, 48.747 per cent of which = 45.318 cc. was in the left side. The analysis at the close showed a total of 92.968, an

amount equivalent to 52.784 cc. being from the left side. The gain at the anode, 7.464 cc., is equivalent to 80.357 mg. of silver. The deposit in the silver voltameter was 152.42 mg., and the velocity of the anion NO_3 is therefore $\frac{80.357}{152.42} = 0.527$. The time of the electrolysis was six hours, and the current, therefore, was equivalent to 0.00631+ amperes.

MEASUREMENTS OF THE VELOCITIES.

Silver Nitrate in Water.

At the beginning of the investigation several experiments were made in which the electrolysis was allowed to proceed for only a short time, and the results showed a higher velocity of the anion than was obtained when the electrolysis was maintained for a longer period. A large number of tests were then made and similar results were obtained, though these differed quite widely among themselves. Though the change in concentration caused by the current was small and the analytical errors therefore large, they were not sufficient to account for the differences found. It was, therefore, apparent, that the cause was to be sought in the apparatus used. The form then employed was a modification of that finally adopted, the limbs being of full size throughout the entire length and the stoppers being at the top. The electrodes were at the ends of glass tubes, which were inserted through the stoppers and extended to the bottom of the limbs, and in order to secure the adjustment of the level of the liquid in the two limbs it was necessary, at the close of the experiment, to slightly raise the stoppers in order to permit the entrance of the air. The experiments being repeated with the later form of apparatus, no difference in the results was obtained within the limits of the experimental errors. The cause of the discrepancy is probably due to the fact that in the first form of apparatus it was impossible to secure an accurate adjustment of level in the two limbs, at the close of the electrolysis, an error being thus introduced, which, though small, was sufficient to account for the differences noted.

In the following tables t denotes the temperature of the

bath ; m the concentration of the solution in gram-equivalents per liter ; c the change in concentration expressed in milligrams ; v the amount of silver in milligrams, deposited in the voltameter ; a the velocity of the anion ; while I and II designate the apparatus employed ; A and B the voltameters ; and L and R indicate whether the anode was in the left or right side of the apparatus.

	$t.$	$m.$	$c.$	$v.$	$a.$	
1	0°	0.1	71.486	132.02	0.5414	I, B, R.
2	0°	0.1	66.200	122.43	0.5407	I, A, L.
3	0°	0.1	43.182	79.77	0.5413	II, B, R.
4	47°.9	0.1	80.358	152.42	0.5272	II, A, L.
5	47°.4	0.1	91.425	172.94	0.5286	II, A. R.
6	47°.7	0.1	82.231	155.74	0.5280	I, B, R.
7	29°.1	0.1	27.647	52.04	0.5317	I, A, R.
8	0°	0.025	39.522	73.47	0.5377	I, A, R.
9	45°	0.025	41.955	79.97	0.5246	II, B, L.

The values obtained by other observers under similar conditions are given in the following table :

Hittorf.			Nernst and Loeb.		
$t.$	$m.$	$a.$	$t.$	$m.$	$a.$
19°	0.118	0.526	20°	0.1043	0.528
19°	0.055	0.526	26°	0.0521	0.524
19°	0.024	0.526	26°	0.025	0.522
			0°	0.025	0.538
			26°	0.0105	0.523

Silver Acetate in Water.

In this investigation it was found impossible to secure exact results in concentrated solutions. The analyses showed not only great variations but also that a considerable loss in silver had taken place. A light-brown deposit of silver oxide was formed on both electrodes, though less upon the cathode, due probably to the formation of complex ions. As was noticed by Nernst and Loeb, if the concentration at the anode became considerable the salt crystallized out ; but in most cases this did not interfere with the results.

	$t.$	$m.$	$c.$	$v.$	$a.$	
1	0°	0.025	9.99	26.56	0.3746	II, B, L.
2	0°	0.025	23.386	62.82	0.3723	I, A. L.
3	28°.3	0.025	10.84	28.42	0.3818	I, B, R.
4	45°.4	0.025	30.261	79.55	0.389	I, A, R.
5	44°.7	0.025	29.316	75.24	0.3896	II, B, R.
6	49°.4	0.025	28.969	74.79	0.3873	I, A, R.

The average values as given by these results are

t.	*m.*	*a.*
0°	0.025	0.373
28°.3	0.025	0.382
46°.5	0.025	0.389

The value obtained by Nernst, though at greater dilution, is

t.	*m.*	*a.*
24°.5	0.00972	0.376

while that calculated from the above results for the same temperature is

t.	*m.*	*a.*
24°.5	0.025	0.380+

Silver Nitrate in Alcohol.

In these experiments it was found impossible to make use of the ice- or water-bath, as a rapid diffusion of water took place through the stoppers in spite of every precaution. The experiments were made, therefore, at the temperature of the room, the apparatus being placed in a wooden case having a thick cork lining to prevent, as far as possible, any diffusion that might be caused by air currents. The following results were obtained :

Silver Nitrate in Absolute Alcohol.

	t.	*m.*	*c.*	*v.*	*a.*	
1	19°.5	0.10805	32.518	54.85	0.5928	II, B, L.
2	20°.9	0.10805	30.772	51.93	0.5926	I, A, L.
3	20°	0.10805	23.192	38.94	0.5956	II, B, L.

The average of these values is

t.	*m.*	*a.*
20°	0.10805	0.594

Silver Nitrate in 49.7 Alcohol.

	t.	*m.*	*c.*	*v.*	*a.*	
1	24°.4	0.1	79.626	149.8	0.5316	II, B, L.
2	23°	0.1	79.894	149.9	0.5329	II, A, R.

The average of the values is

t.	*m.*	*a.*
23°.7	0.1	0.5323

While in water the velocity calculated for the same temperature and concentration is

t.	*m.*	*a.*
24°	0.1	0.533

It is evident, therefore, that in solutions of this strength the alcohol exerts little or no influence upon the velocity, while in the pure solvent the velocity of the anion $\overline{NO_3}$ is very markedly increased, that of the cation $\overset{+}{Ag}$ being similarly decreased.

Ionic Velocities and Conductivity.

The investigations of Kohlrausch[1] and Ostwald[2] have demonstrated that the molecular conductivity[3] of an electrolyte increases with the dilution and reaches at a definite limit a maximum value; and where the conductivities have been determined through a sufficient range of dilutions it is possible to calculate this value by extrapolation. Kohlrausch also proved by a comparison of the values obtained by him in a large number of salts, with the relative velocities of the ions as given by Hittorf, that μ_∞ is equivalent to the sum of the two independent constants, which are, in fact, the velocities of the ions at infinite dilutions in terms of conductivity units. Denoting these velocities by A and C we have the equation $\mu_\infty = A + C$. Moreover, the calculation of A and C is simple since $C/A = c/a$; where in the case of potassium chloride (KCl), at 25° $\mu_\infty = 140 = A + C$; and since $c = 0.481$ and $a = 0.509$, $\frac{C}{A} = \frac{0.491}{0.509}$, hence $C = 68.6$ and $A = 71.4$. Moreover, experiment shows that the same law holds good for greater concentrations, being, in all, save in the most extreme concentration, equal to the sum of the velocities.

[1] F. Kohlrausch: Wied. Ann., **26**, 194 (1895).

[2] Ostwald: Lehrb. allg. Chemie, II, 621.

[3] By molecular conductivity is meant the ratio of the conductivity, k, to the concentration in gram-molecules per liter, *i. e.*, $\mu_v = k/m$.

The following table gives the values of the velocities calculated in this manner from the results of the foregoing experiments upon solutions of silver nitrate in water. The conductivities were calculated from those given by Kohlrausch, assuming that the temperature coefficients for different dilutions, as determined between 18° and 25°, hold good between 0° and 48°. t denotes the temperature, m the concentration in gram-molecules per liter, μ_v the conductivity in mercury units, A the velocity of the anion, and C that of the cation. The values of the relative velocities for 18° and 25° were calculated from those obtained at the other temperatures.

t.	m.	μ_v.	A.	C.
0°	0.1	641	347	294
18°	0.1	887	475	412
25°	0.1	1018	542	476
29°.1	0.1	1061	564	497
47°.7	0.1	1448	764	684
0°	0.025	732	394	338
18°	0.025	979	521	458
25°	0.025	1128	598	530
45°	0.025	1617	848	769

The values obtained by Nernst, under similar conditions, are as follows :

t.	m.	μ_v.	A.	C.
25°	0.1	1018	540	478
0°	0.025	732	394	338
25°	0.025	1128	591	537

For solutions of silver acetate in water and for solutions of silver nitrate in alcohol, it is impossible to make exact calculations, as the conductivities have not been determined for concentrations greater than $m = 0.007$ in the case of the acetate, and $m = 0.025$ in the case of the nitrate.

The absolute velocities were calculated in the usual manner from the formulas of Hittorf and Kohlrausch. The following results were obtained, C' being the absolute velocity of the cation and A' that of the anion.

Silver Nitrate in Water.

t.	m.	A'_{NO_3}. Cm. per sec.	C'_{Ag}. Cm. per sec.
0°	0.1	0.000382	0.000324
18°	0.1	0.000522	0.000455
25°	0.1	0.000597	0.000524
29°.1	0.1	0.000621	0.000548
45°	0.1	0.000842	0.000753
0°	0.025	0.000434	0.000372
18°	0.025	0.000574	0.000505
25°	0.025	0.000659	0.000584
45°	0.025	0.000934	0.000848

The values obtained by Nernst and Loeb are as follows:

t.	m.	A'_{NO_3}. Cm. per sec.	C'_{Ag}. Cm. per sec.
25°	0.1	0.000595	0.000527
0°	0.025	0.000434	0.000373
25°	0.025	0.000651	0.000592

showing a very close agreement with those given above.

It is interesting to compare the result deduced for the cation C' at 18° and $m = 0.1$ with that obtained by W. Whetham,[1] who measured directly the velocities in centimeters by observing the movement of the precipitate formed at the boundary between solutions of silver nitrate and sodium nitrate, the latter containing just enough sodium chloride to make the precipitate visible. He found:

t.	m.	C_{Ag}.
18°	0.1	0.000488 cm. per sec.

The difference between this and that already given, 0.000455, is within the range of experimental errors.

Conclusions.

From the experiments here described we may conclude:

1. That the wide differences observed between the velocities at 0° become less as the temperature rises, the velocities tending towards equality, as has been observed by others.

2. That the effect of decrease in concentration is in the same direction as that of increase in temperature.

[1] Phil. Trans., (1895), Vol. 186A.

3. That the velocities are largely dependent upon the nature of the solvent.

In conclusion, I wish to express my indebtedness to the late Professor Rowland and to Professor Ames for advice during the progress of this work; to Professor Morse for suggestions in regard to themethod of analysis; and to Dr. H. C. Jones, at whose suggestion this investigation was undertaken, for the preparation and purification of several of the compounds used, and for advice and assistance during the progress of the experiments.

JOHNS HOPKINS UNIVERSITY,
June, 1897.

Supplementary Note.

The work described in the above paper was finished in June, 1897, and a brief account of it was published in the Johns Hopkins University Circular, Vol. 16, No. 130, p. 45, June, 1897. Since that time a number of papers[1] have appeared bearing upon this problem. Of these investigations, by far the most extensive is that of Bein. Indeed, this is the most elaborate investigation on the direct measurement of the relative velocity of ions that has been carried out since the time of Hittorf. Bein describes in all five forms of apparatus with various means of separating the cathode solution from the anode solution. In one of these the two solutions are separated by means of a pinch-cock. In another, the solutions are drawn off in a manner similar to that employed in the apparatus of Nernst and Loeb. In still another form the separation is effected by changing the mercury level, and so on.

On examining the apparatus devised and used by Bein, one is impressed by its comparative complexity. Indeed, three out of the five forms which he constructed are very complex. The question arises whether such a high degree of complexity is necessary in an apparatus for effecting electrolysis and the subsequent separation of the solutions.

[1] K. Hopfgartner: Ztschr. phys. Chem., **25**, 115; G. Kummel: Wied. Ann., **64**, 655; V. Gordon: Ztschr. phys. Chem., **23**, 469; W. Bein: *Ibid.*, **27**, 1. **28**, 439; O. Masson: *Ibid.*, **29**, 501, Phil. Trans., **192**, A. 331; F. Kohlrausch: Wied. Ann., **66**, 785; A. A. Noyes: Ztschr. phys. Chem., **36**, 61; B. D. Steele: J. Chem. Soc., **69**, 414.

The following results were obtained by Bein for silver nitrate and silver acetate. The velocities given are those of the anion in each case:

$AgNO_3$. Velocity of $\overline{NO_3}$.		CH_3COOAg. Velocity of $\overline{CH_3COO}$.	
At 74°	0.518	At 24°	0.413
" 79°	0.516	" 24°	0.414
" 94°	0.507	" 49°.5	0.412
" 96°	0.518	" 96°.5	0.435
		" 96°.0	0.442

These results differ considerably from those found by me. The velocity of $\overline{NO_3}$ is smaller, but the temperatures used were higher. This is what we should expect, since it is well known that increase in temperature diminishes the relative velocity of the swifter ion. The above values for $\overline{CH_3COO}$ are higher than those found by me, and the temperatures are higher. This is again what should be expected, since rise in temperature increases the relative velocity of the slower ions.

Kummel and Masson have each devised new forms of apparatus for studying the velocities of ions. These resemble in principle the forms which had already been used.

Quite recently Noyes has described an apparatus which differs considerably from any form previously used. It consists essentially of two U-tubes attached by rubber tubing. The electrodes are inserted into the long arms of the tubes, which are filled with the solution to only a few centimeters above the bend. After the electrolysis was ended, the solution was separated into three portions by inserting a pipette attached to a suction pump.

Steele's method, in general, resembles Masson's, but differs from it in that the measurements are made in water and not in gelatin, and the use of a colored ion as indicator is not necessary.

JUNE, 1901.

Contributions from the Chemical Laboratory of the Massachusetts Institute of Technology.

XXXIII.--THE CONDENSATION OF CARBON TETRACHLORIDE WITH HALOGEN DERIVATIVES OF BENZENE BY MEANS OF THE FRIEDEL AND CRAFTS REACTION.

By James F. Norris and Erik H. Green.

It has been shown[1] recently that the action of carbon tetrachloride on benzene in the presence of aluminium chloride results in a nearly quantitative yield of triphenylchlormethane. As the reaction is a very smooth one, we have studied the condensation of carbon tetrachloride with a number of benzene derivatives, expecting in this way to obtain the corresponding derivatives of triphenylmethane. Among the compounds investigated were certain halogen derivatives of benzene. The behavior of these compounds in the condensation was unexpected; and as the results are of some interest in connection with the application of the Friedel and Crafts reaction to aryl halides, they are given here in the form of a preliminary paper.

Chlorbenzene reacts readily at room temperature with carbon tetrachloride in the presence of aluminium chloride. The product of the reaction was not the expected triphenylmethane derivative, but proved to be an oil, which distilled under diminished pressure without decomposition, and when heated with water lost 2 atoms of chlorine and was converted into 4,4′-dichlorbenzophenone. The yield was good, and the only by-product was a low-melting substance, probably an isomer, which was formed in small quantity. In the condensation but two of the chlorine atoms in the carbon tetrachloride entered into the reaction, and, as a result, a ketone chloride was formed. With benzene, on the other hand, and according to

[1] Gomberg: J. Am. Chem. Soc., **22**, 752; Norris and Saunders: This Journal, **25**, 54.

Gomberg and Voedisch,[1] with toluene also, the reaction consists in the removal of three chlorine atoms.

The condensation with brombenzene is analogous to that with chlorbenzene and furnishes a ready means of obtaining a good yield of 4,4′-dibrombenzophenone chloride. This chloride yields the corresponding ketone, when heated with dilute alcohol, which is identical with the compound obtained by Hoffmann[2] from *p*-brombenzoyl chloride and brombenzene. 4,4′-Dibrombenzophenone chloride could not be distilled even under diminished pressure.

The preparation of symmetrical halogen-substituted ketones from carbon tetrachloride seems to be more efficient than the process of condensing substituted benzoyl chlorides with halogen derivatives of benzene. The latter method, according to Schöpff,[3] gives poor yields, and the high temperature at which the reaction is carried out occasions deep-seated changes through the action of the aluminium chloride present. Schöpff heated a mixture of *p*-brombenzoyl chloride and brombenzene with aluminium chloride to 120° to 130° and obtained a small yield of 4,4′-dibrombenzophenone, together with some monobrombenzophenone. The work of Hoffmann[4] also shows that the preparation of di-iodobenzophenone by this process is complicated by the formation of some iodobenzophenone. That these complications are due to the destructive action of aluminium chloride at slightly elevated temperatures is shown by the work of Dumreicher,[5] who found brombenzene and aluminium chloride to react under these conditions to form benzene and dibrombenzene along with ill-defined products.

With carbon tetrachloride, however, the condensation of monohalogen derivatives of benzene proceeds at room temperature, without the formation of resinous by-products. Preliminary experiments show that the alphyl carbon atom, as is general in the Friedel and Crafts reaction, takes the position para to the halogen in the benzene ring.

[1] J. Am. Chem. Soc., **23**, 177.
[2] Ann. Chem. (Liebig), **264**, 163.
[3] Ber. d. chem. Ges., **24**, 3766.
[4] *Loc. cit.*
[5] Ber. d. chem. Ges., **15**, 33.

The application of the reaction to benzene derivatives containing two halogen atoms was studied carefully, as the preliminary results proved to be at variance with the observations of previous workers who have attempted to condense such compounds by means of aluminium chloride. Heretofore the results have always been abnormal. Methyl chloride, according to Leroy,[1] does not condense with *p*-dibrombenzene, and its unusual action with *o*-dichlorbenzene has been fully described by Friedel and Crafts.[2] The work of Gattermann and Römer[3] on the substituted thiophenes has shown that while the monosubstituted derivatives react normally with acetyl chloride, the dihalogen compounds are either inert or else give rise to a number of complicated compounds, which would not be expected as the result of the reaction. The same observers tried, in vain, to condense acetyl chloride with *p*-dibrombenzene, and Schweitzer[4] was equally unsuccessful with diiodobenzene and with 1,4-dibromnaphthalene. In fact, it has been taken as a rule that the Friedel and Crafts reaction is not applicable to dihalogen compounds.

We have found, however, that when carbon tetrachloride is used as the fatty halide, a condensation takes place normally between it and dichlorbenzene. When a solution of these compounds in carbon bisulphide is treated with aluminium chloride and is heated gently, reaction takes place. The aluminium compound which separates yields, after decomposition with water, a compound which is obtained quite pure after a single crystallization. This substance proved to be the chloride of symmetrical 2,5,2′,5′-tetrachlorbenzophenone. When boiled with dilute alcohol it lost 2 atoms of chlorine and was converted into the corresponding tetrachlorbenzophenone. The structure of the compound follows from its formation from *p*-dichlorbenzene, for in this case there is no opportunity for isomerism.

The easy formation of this tetrachlorketone chloride is the

[1] Bull. Soc. Chim., **48**, 214.
[2] Ann. chim. phys., (6), **10**, 471.
[3] Ber. d. chem. Ges., **19**, 691.
[4] *Ibid.*, **24**, 552.

more interesting on account of the fact that Schöpff[1] has shown that the halogen derivatives of the benzene hydrocarbons react with acyl chlorides only when the position para to the aryl halogen is unoccupied. He found, for example, that *p*-bromtoluene does not react with acetyl chloride, while *o*-bromtoluene reacts readily. Our experiments show that when carbon tetrachloride is used the Friedel and Crafts reaction proceeds normally not only when the position para to the aryl halogen is filled, but even when the only position which the alphyl carbon atom can enter is ortho to a halogen in the benzene ring.

Paradibrombenzene reacts with carbon tetrachloride and aluminium chloride. The compound which is formed is now under investigation. Symmetrical tribrombenzene does not react under the same conditions. After heating the reaction-mixture for twenty-five hours all of the tribrombenzene was recovered. Symmetrical tetrabrombenzene behaved similarly. In order to learn whether the stability of these two compounds is due to the accumulation of halogen atoms about the benzene ring, or to the fact that the entering carbon atom must take a position ortho to two halogen atoms, the behavior of unsymmetrical tribrombenzene will be investigated.

Preliminary experiments on the action of carbon tetrachloride in the presence of aluminium chloride on anisol, mesitylene, dimethylaniline, and naphthalene show that these substances readily react at ordinary temperatures. The products will be further studied.

EXPERIMENTAL.

4,4',-*Dichlorbenzophenone Chloride.*—In the first experiments one molecular quantity of carbon tetrachloride and three and one-half of chlorbenzene were used. As but 2 molecules of chlorbenzene were found to enter into the reaction the proportions of the reaction-mixture were accordingly changed. The same product was obtained when the reaction was allowed to proceed at room temperature and when the mixture was heated to the boiling-point of carbon disulphide.

[1] *Loc. cit.*, p. 3769.

The preparation was carried out as follows: 20 grams of carbon tetrachloride and 28 grams of chlorbenzene were dissolved in 150 cc. carbon disulphide. To this mixture 30 grams of aluminium chloride were added. A reaction began in about half an hour, and soon hydrogen chloride was evolved in large quantities. After twenty-four hours the solution was decanted from the double aluminium compound, which was washed with carbon disulphide to remove the excess of chlorbenzene. The residue was decomposed with ice and extracted with carbon disulphide. The filtered extract was dried over calcium chloride and fractionated under a pressure of 38 mm. After the removal of the solvent, the product distilled over at 205°–220°, and a small quantity of a tarry substance remained in the flask. The oil, when redistilled at 38 mm., boiled at 217°–219° (corr.). About 14 grams were obtained. A large amount of chlorbenzene was recovered, and it was evident that the reaction was not complete. If the reaction had proceeded longer a better yield would probably have resulted. The substance was a pale-green, very viscid liquid of peculiar odor. It was soluble in carbon disulphide, petroleum ether, and ether, slightly soluble in cold alcohol, and insoluble in water. It dissolved readily in hot, dilute alcohol and 4,4′-dichlorbenzophenone crystallized out in brilliant plates on cooling. Cold concentrated sulphuric acid dissolved the oil with the evolution of hydrogen chloride; on dilution the ketone was precipitated.

The high-boiling residue from the fractionation of the substance was extracted with hot alcohol; on cooling, crystals of the ketone separated, together with a small quantity of long, colorless needles, which melted at 66°–68°. The amount of this substance obtained was too small to warrant further study. The 4,4′-dichlorbenzophenone chloride was analyzed by boiling it with dilute alcohol and titrating the hydrochloric acid formed. 23.43 per cent of acid was obtained instead of 23.20, the theoretical amount.

4,4′-*Dichlorbenzophenone*.—This ketone is readily obtained from its chloride by heating with dilute alcohol, or by solu-

tion in concentrated sulphuric acid and subsequent dilution. The product so formed was identified with the ketone obtained by Dittrich[1] by the action of *p*-chlorbenzoyl chloride on chlorbenzene and aluminium chloride. It was readily soluble in carbon disulphide, chloroform, ether, glacial acetic acid, and hot dilute alcohol or acetone. When a solution of the ketone in concentrated sulphuric acid is allowed to stand some time in the air, the ketone crystallizes out in plates. It crystallizes from hot dilute alcohol in glistening, pearly plates, which melt at 144°.5. Two determinations of chlorine in the ketone gave 28.21 and 28.29 per cent instead of 27.90 per cent.

4,4′-*Dibrombenzophenone.*—A mixture of 20 grams carbon tetrachloride, 40 grams brombenzene, 30 grams aluminium chloride, and 150 cc. carbon bisulphide was allowed to react at room temperature for two days. The reaction-product was decomposed and treated as described above under the analogous chlorine compound. The dark oil so obtained decomposed with evolution of bromine when an attempt was made to distil it under diminished pressure. It resembled in its chemical properties the analogous chlorine derivative, and from it was obtained 4,4′-dibrombenzophenone, which was identified with the compound obtained by Hoffmann from brombenzoyl chloride and brombenzene. The ketone melts at 171°–172°. It is soluble in carbon bisulphide, chloroform, benzene, and in hot alcohol or acetone; it is less readily soluble in ether and acetic acid. A determination of bromine gave 47.22 per cent; the theory requires 47.06 per cent.

2,5,2′,5′-*Tetrachlorbenzophenone Chloride.*—20 grams *p*-dichlorbenzene and 6.5 grams carbon tetrachloride were dissolved in 100 cc. carbon bisulphide and 10 grams aluminium chloride were added. There was no reaction at room temperature. After the mixture had been boiled for ten hours the carbon disulphide was filtered off and evaporated to crystallization. Fifteen grams of *p*-dichlorbenzene were recovered. The aluminium compound was decomposed with water and extracted with carbon disulphide. The filtered extract deposi-

[1] Ann. Chem. (Liebig), **264**, 175.

ted, on evaporation, a mass of brilliant, colorless crystals. As the compound is very soluble in carbon disulphide it was found that crystals could be obtained more readily by adding alcohol and then by boiling to get rid of most of the carbon disulphide. On cooling, the substance crystallizes out in leaflets. In this way 5 grams of 2,5,2′,5′-tetrachlorbenzophenone chloride, melting at 173°–174°, were obtained. The yield was good since but 5 grams of dichlorbenzene entered into the reaction. In later experiments the quantity of dichlorbenzene which was used up was materially increased by heating the reaction-mixture for two days and adding small portions of aluminium chloride from time to time.

The tetrachlorbenzophenone chloride dissolves easily in chloroform, benzene, and acetone, less readily in ether, and very slightly in hot alcohol and concentrated sulphuric acid. An analysis gave the following results:

0.2333 gram substance gave 0.5306 gram AgCl (Carius).

	Calculated for $(Cl_2C_6H_3)_2CCl_2$.	Found.
Cl	56.80	56.46

The ketone chloride is converted into the corresponding ketone by boiling with dilute alcohol, or more quickly by heating in a sealed tube to 130° with dilute alcohol or water. The chloride is much more stable than the two ketone chlorides described above.

2,5,2′,5′-Tetrachlorbenzophenone. — Several grams of the ketone chloride were boiled with dilute alcohol for four hours under a return condenser. The solution was filtered hot from unchanged chloride. On cooling, large, feathery plates separated, which on recrystallization from alcohol melted sharply at 128°. The original mother-liquor was found to contain hydrochloric acid. The crystals were colorless and very brilliant. They dissolved readily in hot alcohol, petroleum ether, carbon disulphide, benzene, acetone, ethyl acetate, and chloroform; less easily in cold alcohol and concentrated sulphuric acid. The analysis agreed with the composition calculated for tetrachlorbenzophenone.

0.2560 gram substance gave 0.4611 gram AgCl.

	Calculated for $(Cl_2C_6H_3)_2CO$.	Found.
Cl	44.37	44.53

The ketone was obtained more rapidly by heating the chloride with a mixture of 1 part alcohol and 3 parts water in a tube, at 130°, for two hours. The ketone was separated from any unchanged chloride by treatment with hot alcohol in which the chloride is almost insoluble.

BOSTON, MASS., August 1, 1901.

XXXIV.—ON THE PREPARATION OF TRIPHENYLMETHANE.

BY JAMES F. NORRIS AND GRACE MACLEOD.

A short time ago[1] the results of some work done in this laboratory on the condensation of carbon tetrachloride with benzene in the presence of aluminium chloride were published. Friedel and Crafts[2] and E. and O. Fischer[3] had previously studied this reaction and had obtained triphenylmethane as the result. We obtained, however, a nearly theoretical yield of triphenylchlormethane. The difference in the result was due to the fact that the earlier workers had distilled the reaction-product, whereas we had purified it by crystallization, care being taken to prevent any decomposition.

As it was evident that, in this case, distillation had resulted in the complete destruction of the true product of the reaction, it seemed important to examine other condensations in such a way that the actual result of the reaction might be isolated. We first undertook to study carefully the preparation of triphenylmethane from chloroform, benzene, and aluminium chloride.

The crude reaction-product has always been distilled in this preparation, and beside the expected hydrocarbon other compounds are obtained in appreciable quantities. It has been observed by those who have studied this reaction, that at a

[1] Norris and Saunders: This JOURNAL, **23**, 54.
[2] Compt. rend., (1877), p. 1453.
[3] Ann. Chem. (Liebig), **194**,

certain point in the distillation, hydrochloric acid is given off, and at the end a carbonaceous residue is left. The reaction-product evidently contains a halogen compound, which is decomposed during the distillation.

From an examination of the cases in which the Friedel and Crafts reaction has been applied to polyhalogen compounds, and the products of the condensation have been isolated by crystallization, it was evident that the normal reaction consisted in only a partial elimination of the halogen atoms. Carbon tetrachloride gives with benzene triphenylchlormethane, with toluene tritolylchlormethane, and with *p*-dichlorbenzene, tetrachlorbenzophenone chloride. Phenyl iodidedichloride gives diphenyliodonium chloride, and the unsymmetrical chloride of *o*-sulphobenzoic acid gives a compound which contains one chlorine atom. It seemed of importance, therefore, to determine whether the conclusion is warranted which is indicated by these examples, namely, that in the Friedel and Crafts reaction polyhalogen compounds do not lose all of their halogen atoms during the condensation. The investigation of this question appeared worth while, as in many cases compounds containing two or more halogen atoms linked to the same carbon atom have given abnormal results when condensed with hydrocarbons, *e. g.*, unsymmetrical tetrabromethane gives symmetrical tetraphenylethane together with a number of unexpected products.

A condensation of chloroform and benzene was effected according to the directions of Friedel and Crafts. The reaction-mixture was boiled in order to bring about the elimination of as much hydrogen chloride as possible. The aluminium addition-product was decomposed carefully with ice in the presence of a large quantity of carbon disulphide in order to prevent any decomposition of the true product of the reaction. The solution so obtained was dried over calcium chloride and was allowed to evaporate in a current of air. After most of the solvent was removed a dark-brown oil remained, from which crystals separated slowly. At the end of about six weeks, over one-half of the product had been obtained in

crystalline condition, and an oil was left which did not crystallize on further standing. All attempts to obtain crystals from this oil were without success. The result of the experiment was surprising as not a trace of triphenylmethane was found. The crystals proved to be triphenylcarbinol, and the oil was readily soluble in cold concentrated sulphuric acid, in which triphenylmethane is insoluble.

On account of this unexpected result the experiment was repeated a number of times under different conditions, but in every case with the same results. In order to find out if, in our experiments, the condensation had taken place in the usual way, the product of a reaction was divided into two portions,—about three-fourths was subjected to distillation and one-fourth was crystallized and tested for triphenylmethane. When distillation was used a good yield of the hydrocarbon, together with some diphenylmethane, was obtained. Crystallization produced the same results as before. These experiments showed conclusively that the triphenylmethane which is obtained in this reaction is the product of the destructive distillation of a mixture of substances which does not contain the hydrocarbon.

We, accordingly, undertook to discover by what reactions triphenylcarbinol was formed, and to determine what substances were present in the uncrystallizable oil. The simplest explanation of the formation of the carbinol appeared to be that at first the three chlorine atoms in the chloroform were replaced by phenyl groups in the usual way, and then the resulting triphenylmethane was converted into the carbinol by the oxygen of the air in the presence of aluminium chloride. The oxidation of a hydrocarbon to a hydroxyl compound in this way was shown to be possible by Friedel and Crafts, who converted benzene into phenol by the action of oxygen in the presence of aluminium chloride. With triphenylmethane, however, the phenomenon is different in that the hydrogen oxidized is joined to a methane carbon atom.

The action of oxygen on the hydrocarbon and aluminium chloride was accordingly studied. Friedel and Crafts have

already noted the fact that the chloride decomposes triphenylmethane in part when the substances are heated together. Heated at 120° for ten minutes, the hydrocarbon becomes resinous and yields some benzene. If warmed for ten hours, at 70°, with 7.5 parts of benzene and 1 part of the chloride, diphenylmethane is formed. No details of the experiments are given, but it is probable that the reaction-products were distilled.*

In the experiments made by us, a solution of triphenylmethane in carbon disulphide was treated with aluminium chloride in the cold. The chloride slowly extracted the hydrocarbon from the solvent and was thus converted into a brown, gummy mass. After standing about two days this mass was separated and was washed a number of times with carbon disulphide to remove any triphenylmethane that had not reacted with the chloride. The intermediate product was decomposed by ice and the organic part was extracted. On evaporation of the solvent a resinous substance was obtained from which no crystalline material could be separated. The product dissolved in concentrated sulphuric acid in the cold, and therefore contained no triphenylmethane. The experiment showed that even in the cold, triphenylmethane is decomposed by aluminium chloride.

A second series of experiments was made under the same conditions as the first, with the exception that air was drawn through the mixture. After careful decomposition the organic part was taken up in carbon disulphide, and the solution was dried and evaporated in a current of air. The product resembled that obtained in the first experiments from chloroform, benzene, and aluminium chloride. Crystals of triphenylcarbinol separated in appreciable quantity and a thick oil, soluble in sulphuric acid, was left. It is evident from this experiment that triphenylmethane is converted into triphenylcarbinol and a product that cannot be brought to crystallization when it is treated with aluminium chloride and oxygen.

A portion of the reaction-product of this last experiment was treated with sulphuric acid after the solvent had been re-

moved by evaporation. Solution took place with the evolution of hydrogen chloride. When this solution was poured into water, triphenylcarbinol was formed. The evolution of hydrogen chloride during the solution of the reaction-product was evidence of the presence of some organic halogen compounds in the mixture. This compound was shown to be triphenylchlormethane. An experiment showed that aluminium chloride converts triphenylcarbinol into triphenylchlormethane. A solution of the pure carbinol in carbon disulphide was treated with the chloride. The dark gummy compound which was formed was decomposed by ice and then extracted. The solution so formed was dried and evaporated in a stream of dry air. Crystals were obtained which melted indistinctly near the melting-point of triphenylchlormethane. It was impossible to purify the substance by recrystallization as the chloride is readily converted into the carbinol. The impure material was accordingly boiled with alcohol to convert any triphenylchlormethane present into the corresponding ether. A sufficient quantity of the ether was obtained in a pure condition and identified. The work, therefore, proves that triphenylmethane is converted by air, in the presence of aluminium chloride, in part into triphenylcarbinol, and that this, in turn, is to some extent changed into triphenylchlormethane.

An attempt was next made to determine what substances were present in the oil which was formed along with triphenylcarbinol from chloroform and benzene. This oil, which was present to the extent of nearly 50 per cent, could not be brought to crystallization in any way. It seemed possible at first that it might contain diphenylmethane, triphenylmethane, diphenylchlormethane, diphenylcarbinol, triphenylchlormethane, benzhydrol ether, or triphenylcarbinol. It dissolved in sulphuric acid in the cold with slight evolution of hydrogen chloride. When this solution was poured into a large volume of water, a precipitate was formed in small quantity. This precipitate was shown to be triphenylcarbinol.

The action of cold sulphuric acid on the substances men-

tioned above was studied. The hydrocarbons were insoluble after standing a day in contact with the acid. Triphenylcarbinol dissolved readily and was precipitated quantitatively on dilution. Triphenylchlormethane dissolved with evolution of hydrogen chloride and gave the carbinol on dilution. Diphenylcarbinol and benzhydrol ether were insoluble, and diphenylchlormethane dissolved with evolution of hydrogen chloride. The test with sulphuric acid showed that the chief constituent of the oil was not one of the substances enumerated above.

On account of the fact already mentioned that a number of polyhalogen compounds give products in this reaction which contain halogen, a search was made for diphenylchlormethane. A portion of the heavy oil was distilled with steam. A few drops of a light-yellow oil were found in the distillate. When treated with ice this substance solidified to crystals which melted at 22° to 23°. The compound which contained chlorine, upon standing some days in contact with water, changed into another free from halogen, and hydrochloric acid was found in solution. Diphenylchlormethane might act in this way, but Engler,[1] who has described this compound, gives its melting-point as 14°. A sample prepared by us by the method of Engler melted at 18° to 20°. It is possible that the substance was a small quantity of diphenylchlormethane which distilled over without decomposition by the steam. Nearly the entire amount of the original oil was apparently unchanged. It was soluble in sulphuric acid as before, but the distillation flask contained a trace of hydrochloric acid.

To test the behavior of diphenylchlormethane with steam, about 2 grams of it were distilled. A very small quantity of diphenylcarbinol passed over with the steam. The product left in the distillation-flask was shown to be benzhydrol ether, which was found to be insoluble in sulphuric acid. The original oil, therefore, could not have contained more than a trace of diphenylchlormethane.

These experiments show that the oil was, in all probability,

[1] Ber. d. chem. Ges., 7, 1128.

a complex condensation-product. As it could not be purified it was studied, side by side, with the similar oil obtained by the action of air on a mixture of triphenylmethane and aluminium chloride. Both oils dissolved in sulphuric acid, and the solutions so formed exhibited a strong fluorescence when diluted. These solutions appeared to be identical as far as the experiments were carried. They were heated to boiling, neutralized with barium carbonate, and filtered. The filtrates showed a beautiful blue fluorescence and gave barium salts on evaporation. These salts were soluble with great difficulty, even in boiling water. As no crystals could be obtained and we had no proof that the substances were chemical individuals, they were not studied farther.

In conclusion, it appears from the work that the process by which triphenylmethane is obtained from chloroform is a very complex one, in which a number of unexpected reactions take place. The hydrocarbon is probably produced as a result of the reduction of the intermediate product, triphenylcarbinol, by the uncrystallizable oil, which is completely decomposed during the distillation.

The results also indicate that a thorough study of the condensations of other polyhalogen compounds with hydrocarbons in the presence of aluminium chloride, would probably furnish an explanation of the abnormal results which have been obtained heretofore in nearly every case.

BOSTON, MASS., August 1, 1901.

Contributions from the Chemical Laboratory of Bryn Mawr College.

ON SOME COMPLEX COMPOUNDS OF THALLIUM, AND THE CONSTITUTION OF DOUBLE SALTS.

BY ALLERTON S. CUSHMAN.

I.

In a recent number of this JOURNAL[1] there was published a paper by the writer, entitled "On Some Isomeric Compounds of Thallium, and the Constitution of Double Salts." This

[1] This JOURNAL, 24, 3.

paper bore the date of acceptance for publication of May 15th. In the July number of the *Zeitschrift für anorganische Chemie* appeared a comprehensive article by Richard Jos. Meyer, entitled "Beiträge zur Kenntnis des dreiwerthigen Thalliums," which bore the date of acceptance of May 29th. In so far as these two papers cover the same ground, there would have been no question of priority were it not for the fact that much of the work presented by Meyer had been carried out by C. Wiegand, and published by him as a dissertation, entitled "Über Halogenverbindungen des Thalliums" (Berlin, 1899). As there was at that time no index to dissertational literature, it is not surprising that the author was quite unaware that he had been in a measure anticipated in the field. The priority of Wiegand in having first prepared some of the chlorobromides and bromochlorides has been conceded by the author in a note to the "Chemiker Zeitung."[1] The responsibility, however, of the claim for isomerism, as well as the actual existence and constitution of some of the compounds, still remains with the present writer. After the appearance of the above-mentioned papers, it was early pointed out that certain discrepancies in some of the points presented required clearing up. Wherever discrepancies occur in the presentation of matters of scientific fact it is natural to assume that one or the other must be altogether right; there are, however, other possible explanations. In some cases both may be in a sense right and only the interpretation at fault, while, as a final possibility, both may be wrong. It is to be hoped that any discrepancies that come under discussion here may be considered as falling under the second case.

The study of sesquihalogen compounds is alluring on account of the promise it holds out of shedding light on the much vexed question of inorganic structure, but, on the other hand, it is surrounded with great experimental difficulties. The ease with which these compounds undergo hydrolysis or decomposition, their hygroscopic nature, their mixed equilibria in relation to water of crystallization and state of oxidation, and their generally complex nature, present dangerous pitfalls to the investigator.

[1] Chem. Ztg., (1900), I, 3.

Perhaps the most serious divergence of view between Meyer and the writer lies in the fact that the former has been led to the belief that, in solution, thallic chloride behaves as though it had the structure $(TlCl)Cl_2$. Such a view is not only fatal to certain facts and theories already put forward, but also casts doubt upon a great mass of analytical data, by implying that the chlorine in thallic chloride cannot be completely precipitated by silver nitrate. As a result of previous and recent work the author is of the opinion that such a view is untenable, and that Meyer was led to it by a misinterpretation of certain analytical results. Since this point is of vital importance, it will now be necessary to examine the evidence. In order that the facts which led Meyer to form his opinion may be fully presented, it is necessary to quote him verbatim: "Interessant ist das Verhalten des Thalliumchlorids in stark salpetersaurer Lösung, in einer Lösung also, in der die Hydrolyse stark zurückgedrängt oder ganz aufgehoben ist. Eine Reihe von Fällungsversuchen hat nämlich gezeigt, dass in der salpetersauren Lösung des Thalliumchlorids sowie seiner Kaliumchloriddoppelsalze das Chlor durch Silbernitrat in der Kälte nur teilweise gefällt wird. * * * * Ich wurde zuerst auf diese Thatsache aufmerksam, als ich versuchte, das Chlor in Thalliumchlorid und seinen Doppelsalzen titrimetrisch nach der Volhard'schen Methode zu bestimmen. Hierbei machte man die Erfahrung, dass von den drei Atomen des Chlorids im Maximum nur zwei Atome an Silber gebunden wurden. Diese unvollkommene Fällbarkeit des Thalliumchlorids in salpetersaurer Lösung, lässt bezüglich der Konstitution des Thalliumchlorids zwei Deutungen zu." Here follows some discussion which may be omitted, but which concludes with the words: "Man wird vielmehr annehmen, dass das Thalliumchlorid in nicht hydrolysierter Lösung im Sinne der Formel $(TlCl)Cl_2$ reagiert." Later on in his paper Meyer takes up the point again. After describing the usual Volhard method of procedure, he gives some of his results and shows that in each case only about two-thirds of the total amount of chlorine present is precipitated as silver chloride, and although the results are not quantitative, they seem to

show, at least qualitatively, that one of the three atoms of chlorine is held by the thallium. Finally, Meyer seems to be satisfied with his conclusions for he terminates the discussion by saying: "Ich beabsichtige, diese Fällungsversuche auch auf die Chloride anderer dreiwertiger Metalle auszudehnen."

In repeating Meyer's work with the Volhard method, the fact was confirmed that the percentage of chlorine found never fully represented the total amount present, but it was noted that in each titration a very faint odor of hydrocyanic acid was discernible. Further experiment showed that when solutions containing thallic ions are mixed with solutions of potassium sulphocyanate, potassium cyanide is formed. On referring to the literature it was found that as early as 1858 Pean de Saint-Gilles[1] proved that when solutions of sulphocyanate are treated with oxidizing agents the following reaction takes place:

$$2RCNS + 2H_2O + O_6 = 2RCN + 2H_2SO_4.$$

No one will doubt that thallic ions are strong oxidizing agents, and as potassium cyanide is undoubtedly formed, we have a conclusive explanation of the incomplete precipitation of silver chloride. Meyer himself noted that when first reduced with sulphurous acid the precipitation of the chloride was complete. The author has frequently carefully examined the filtrates from the usual gravimetric determinations, where, of course, no sulphocyanate is used, and no sign of incomplete precipitation of the chlorine has yet been observed. Thus the evidence which supports the writing of thallic chloride as $(TlCl)Cl_2$, as well as all speculation from this point of view, falls to the ground.

At the present time it is difficult to entertain any theory of the structure of inorganic compounds that does not in some way account for the relation of molecules of water of crystallization to the general configuration of the larger molecule.[2]

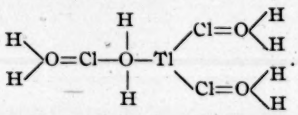
[1] Jsb. Chem., (1858), p. 585.

[2] In the discussion of these theories, a word is constantly needed to distinguish between the smaller and the larger molecule, thus, thallic chloride is stable only when associated with 4 molecules of water. In this paper the "thallic chloride molecule" is used to designate $TlCl_3$, while the stable hydrate $TlCl_3.4H_2O$ will be called the "thallic chloride complex." Where more than one hydrate exists the saturated condition will be so designated, the other being the "lower complex."

In Werner's theory this is done by assuming that molecules of water that cannot be removed by desiccation, or without destroying the molecule, are contained within the nucleus. Thus, as there is some evidence to show that one of the four molecules of water in the thallic chloride complex is differently related to it from the other three, this fact would have to be expressed by Werner's theory as follows:

$$\left(Tl^{III}\begin{matrix}Cl_4\\(H_2O)_2\end{matrix}\right)\begin{matrix}Tl^{III}\\Cl_2\\6H_2O\end{matrix}.$$

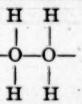
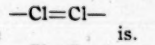
Under the valence theory, as ordinarily written, water of crystallization has never been considered, or accounted for. Granting, however, that oxygen can be quadrivalent, which allows water under certain circumstances to behave as an unsaturated molecule, then we have in hand a valence theory which seems to be capable of giving a satisfactory explanation of a great mass of facts. It is as allowable to assume molecules of water entering into the internal structure of a complex as it is to suppose them in some mysterious way externally attached. To explain the thallic chloride complex, under this theory we should write:



It will be seen that, according to this way of writing, the full saturation value for water, of molecules of the type RCl_3 is six. This is known to be true in the cases of aluminum, iron, and other trivalent atoms, although there is no reason why the fully saturated form should in all cases be the stable one. No thallic chloride complex with six molecules of water has ever been isolated. Kohler has shown[1] that aluminum chloride in water solution, or in any solvent that is known to combine with it, has the molecular weight corresponding to the for-

[1] This JOURNAL, 24, 5.

mula $AlCl_3$, whereas in a saturated indifferent solvent like carbon bisulphide it has the double molecular weight. It will be seen that these facts are accounted for by this theory.

The linkage  is not strong enough to hold the large complex together while the linkage  is. It may be said, in conclusion, that this theory which has grown out of much friendly discussion between Professor Kohler and the author is advanced here simply to the extent that it seems to offer a satisfactory explanation of the constitution of the thallium complexes.

It is not, of course, assumed that this possibility of quadrivalent oxygen is presented here for the first time. It has often been used before, especially to account for the structure of peroxides and persulphates. Moreover, there can be no doubt of the fact that the evidence so far advanced, bearing on such a general theory as the unsaturated character of the water molecule in the formation of hydrated salts, is insufficient. The suggestion is made here only in view of the author's intention to carry the inquiry further.

II.

EXPERIMENTAL PART.

Thallium forms halogen compounds of three definite stages of oxidation, TlCl, Tl_4Cl_6, and $TlCl_3$. As would be expected, the stability of these three compounds is in the reverse order of their respective solubilities. The first two may be called difficultly soluble compounds. Their character and structure have been fully discussed in the author's first paper.[1] Anhydrous thallic chloride, on the other hand, is extremely soluble, not only in water but in most solvents, notably alcohol and ether. It is, however, very hygroscopic and unstable, and it may fairly be doubted if it has ever been prepared in

[1] *Loc. cit.*

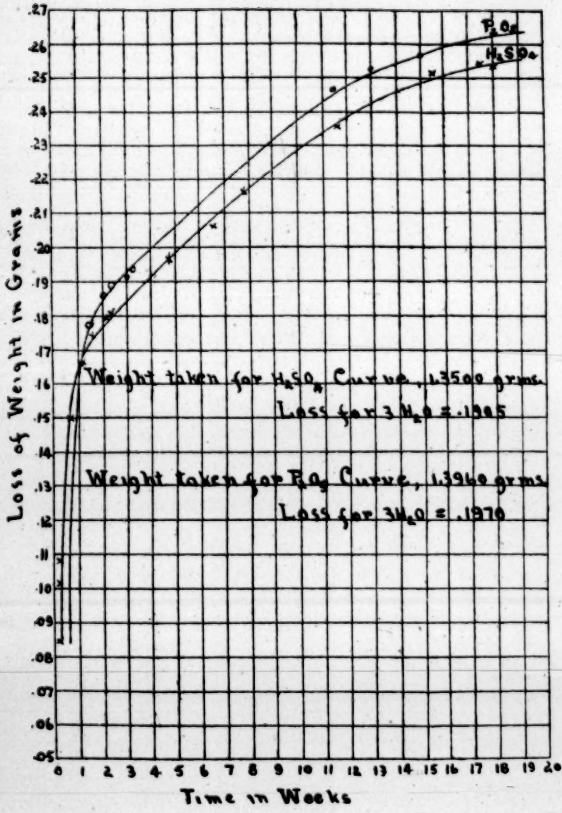
the perfectly anhydrous and unreduced state. Meyer has claimed that he prepared and analyzed it, but repeated efforts made in this laboratory to prepare it in a state fit for analysis and molecular weight determination, have ended in failure. Two water complexes of thallic chloride have been described by Werther,[1] and by Meyer,[2] corresponding respectively to the formulae $TlCl_3.4H_2O$ and $TlCl_3.H_2O$. In order to study the main complex of thallic chloride, a compound was prepared by a method somewhat similar to that adopted by Meyer. Pure thallous chloride was suspended in water, and chlorine was passed in until the oxidation was complete and a clear solution of thallic chloride was obtained. This was evaporated on a water-bath, at 80°, until nearly ready to crystallize, a little more chlorine was then passed in and the mother-liquor left to crystallize spontaneously. In this way beautiful orthorhombic crystals were obtained, some of them measuring more than a centimeter along the major axis. These crystals were crushed, dried by careful pressing between layers of blotting-paper, and analyzed. Meyer describes the crystals which he obtained as being very hygroscopic. These crystals were not at all hygroscopic but, on the contrary, could be left for days exposed to the air, in which case they usually lost a trifle owing to air-drying. In desiccators over various drying agents, the complex begins to lose water at once, as will shortly be shown. The results of the analysis of these crystals is given below, and in order to show that they were probably identical with Meyer's preparation his results are printed in parallel.

1.7730 grams substance yielded 1.8866 grams Tl_2PtCl_6.
1.0053 grams substance yielded 1.1263 grams AgCl.

	Theory for $TlCl_3.4H_2O$.	Found.	Meyer found.
Tl	53.38	53.26	54.36
Cl	27.78	27.70	27.48

Meyer dried his substance before analysis for twenty-four hours over sulphuric acid and caustic potash; that this is not permissible is shown by a glance at the accompanying curve

[1] J. prakt. Chem., 91, 385 (1864).
[2] Ztschr. anorg. Chem., July, 1900.


P_2O_5
H_2SO_4
Loss of Weight in Grams
Time in Weeks
Weight taken for H_2SO_4 Curve, 1.3500 grms.
Loss for $3H_2O$ = .1995
Weight taken for P_2O_5 Curve, 1.3940 grms.
Loss for $3H_2O$ = .1970

shown in the figure. In the experiment from which this curve was plotted, two lots of the paper-dried crystals were weighed in separate watch-glasses and immediately shut up in large

desiccators containing, respectively, sulphuric acid and phosphorus pentoxide. Weighings were made at first every few hours, then every day, and finally every week. The experiment lasted twenty-one weeks, and the rate and order of the loss in weight are fully shown by the plotted curves. The axis of abscissae shows the time in days and weeks, the axis of ordinates, the loss in weight. At the conclusion of the experiment the substances were still very slowly losing weight, owing to loss of chlorine. The complex does not pass the monohydrate condition without losing chlorine. Both Werther and Meyer claim to have isolated the monohydrate, but the author has never succeeded in doing so. The curves both show rather sharp turns, and it may be taken as certain that the last fourth of the water comes off slowly, accompanied by chlorine, but the evidence for the definite monohydrate stage is slight, to say the least. In this connection Meyer says: "Die Entwässerung des Tetrahydrats wurde im Luftbade bei 55° vorgenommen und die Gewichtsabnahme von Stunde zu Stunde festgestellt. Hierbei gehen drei Moleküle Wasser kontinuierlich fort, ohne dass Reduction des Chlorids eintritt. 1.9814 g. nahmen 0.2625 g. ab. Berechnet für 3 Mol. H_2O: 0.2800 g. Bei weiterem Erhitzen beginnt die Schmelze sich gelb zu färben, und es tritt Geruch nach unterchloriger Säure auf. Die Gewichtsabnahme schreitet dann nur äusserst langsam vorwärts. Nach weiterem 2½-stündigem Erhitzen auf 55°, betrug der Verlust nur noch 0.01 g., im Exsiccator blieb das Gewicht dann auch nach 20-stündigem Trocknen konstant."

It will be noted that the existence of a definite monohydrate stage exactly suits the quadrivalent oxygen theory, although it is not demanded by it, since it is equally possible that as soon as the outside protecting water molecules are taken off, chlorine and the last molecule of water begin to leave simultaneously.

The next piece of evidence is interesting and important. Meyer has shown that when the preparation of the thallic chloride complex is attempted in the presence of hydrochloric acid by a method similar to the one described above, a definite crystalline compound is obtained corresponding to the for-

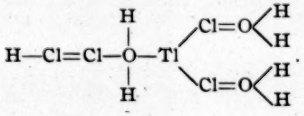
mula $TlCl_3.HCl.3H_2O$. Meyer compares this to a compound of gold, and he writes them respectively,

$$HTlCl_4 + 3H_2O \quad \text{and} \quad HAuCl_4 + 3H_2O.$$

He further points out that they may thus be considered as acid anhydrides which, by combining with 1 molecule of water, lead to the acid hydrates,

$$H_2TlCl_5O \quad \text{and} \quad H_2AuCl_5O.$$

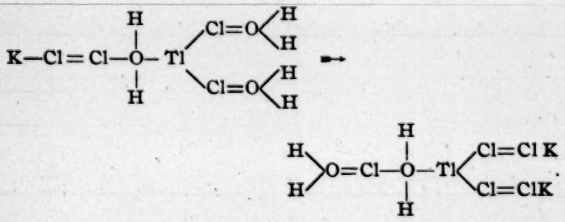
According to the quadrivalent oxygen theory, we might write:



Meyer has described the character of this compound as follows: In dry air it is very stable, but on heating it undergoes reduction with simultaneous loss of water, hydrochloric acid, and chlorine. In solution it reacts strongly acid, and on neutralization with potassium hydroxide and subsequent evaporation it yields crystals, not of the salt $KTlCl_4.xH_2O$, as might be expected, but the definite compound $TlCl_3.2KCl.2H_2O$ according to the equation:

$$2(TlCl_3.HCl) + 2KOH = TlCl_3.2KCl + TlCl_3 + 2H_2O.$$

According to our theory, the rearrangement of the complex is expressed as follows:



It can be inferred from this that the linkage which was strong enough to attach H—Cl to the complex in a certain position is not strong enough to hold the heavier and more basic K—Cl, and thus a redistribution is brought about, the products naturally being the most stable ones possible under the circumstances. Unfortunately, lack of time has not permitted the study of the dehydration of this compound in this laboratory, but the theory would enable us to predict that this compound should lose without reduction but 1 molecule of water. According to Meyer, "dieses Salz giebt beim Erhitzen auf 50° to 60° und im Vakuum-exsiccator, nur ein Molekül Wasser ab. Weitergehende Entwässerung führt auch hier zu Reduction."

One of the peculiarities of thallic chloride and its water complex is the ease with which they dissolve in most solvents, including ether and alcohol. When the thallic chloride complex is dissolved in ether, and the ether evaporated in a current of cold dry air, a crystalline product is obtained. With reference to this product the author finds himself in some disagreement with Meyer. In this laboratory, when dried crystals of the compound $TlCl_3.4H_2O$ were dissolved in pure ether, the liquid divided into two layers—a water solution below and an ether solution above. If the two layers are separated and evaporated, both deposit crystals which resemble each other in appearance and have almost identically the same composition. Meyer, apparently, did not note the formation of the two layers, but obtained, by direct evaporation of the solution of the thallic chloride complex in ether, large, glistening, hexagonal crystals, which he analyzed and represented by the formula $TlCl_3.(C_2H_5)_2O$,—in a word, a thallic chloride complex with 1 molecule of ether of crystallization. Meyer's data are given as follows:

	Calculated for $TlCl_3.(C_2H_5)_2O$.	Found.
Tl	53.09	52.73
Cl	27.64	26.75
Ether	19.27	20.57
	100.00	100.00

It would seem to be a most extraordinary phenomenon if 4 molecules of water in the stable complex could be directly replaced by 1 molecule of ether. If this, indeed, were true, there would be but one way of proving it and that is by making a combustion of the compound. Unfortunately, Meyer made no combustion but determined the ether by difference. It is difficult to see what became of the water in Meyer's preparation, or how his analysis proves the constitution which he publishes, since the compound $TlCl_3.4H_2O$ has a percentage composition almost identical with that of the compound $TlCl_3.(C_2H_5)_2O$. In the author's work, the ether layer was evaporated as described above, until crystallization had progressed to a certain point; the mother-liquor was then poured off and the crystals redissolved in anhydrous ether. This operation was repeated twice, and the final crop of crystals was drained and dried by repeated pressing on blotting-paper. These crystals were analyzed with the following results:

1.5829 grams compound yielded 1.6866 grams Tl_2PtCl_6.

0.8614 gram compound yielded 0.9746 gram AgCl.

	Calculated for $TlCl_3.(C_2H_5)_2O$.	Calculated for $TlCl_3.4H_2O$.	Found.
Tl	53.09	53.38	53.31
Cl	27.64	27.78	27.97

From this analysis it seemed doubtful whether any ether compound was formed at all, and it was quite possible that the water complex was simply crystallizing from the ether solution. Manifestly an organic combustion was necessary to decide the point. A combustion was therefore carefully made, a long layer of lead chromate in the combustion tube insuring the complete absorption of the halogen. The results were as follows:

1.5224 grams substance taken yielded 0.2722 gram H_2O and 0.0585 gram CO_2. From these results it is calculated that 0.0236 gram of ether and 0.2419 gram of water were present in the original substance, and we have for the whole analysis:

Tl	53.31
Cl	27.97
H_2O	15.89
Ether	1.55
	98.72

It is apparent from this analysis that the ether has only very slightly replaced the water in the complex, and indeed it may be doubted whether the water has been replaced at all, since a certain quantity of ether is sure to have been included in the crystals. Meyer states that anhydrous thallic chloride is easily prepared by putting this "ether compound" in a vacuum. A preparation of anhydrous thallic chloride being desired in order that its molecular weight might be determined in different solvents, 20 grams of the substance which had been twice recrystallized from ether were put into a vacuum desiccator over phosphorus pentoxide, and connected with a Sprengel pump. In order to reduce the danger of reduction of the compound, the whole desiccator was placed inside of a refrigerator so that the temperature at no time rose above 18°. For twenty days a fairly good vacuum was maintained. At the end of that time the phosphorus pentoxide had absorbed enough water from the substance to become quite wet. Whatever the compound remaining in the dish may have been, it certainly was not anhydrous thallic chloride. Exposed to the air it was very hygroscopic, but it no longer completely dissolved in anhydrous ether, and gave every evidence of having suffered a partial reduction. Meyer has also described a compound of the formula $TlCl_3.C_2H_5OH$, which he obtained by dissolving the water complex in absolute alcohol, and crystallizing by evaporating the solution *in vacuo*. Since a compound with this formula would have a thallium and chlorine content quite different from that of the water complex, it should be a comparatively easy matter to determine its composition. The author has never made this compound.

The discussion of any further discrepancies between the work of the author and other investigators must be reserved until more work has been done. The pressure of other work has not permitted a thorough reexamination of all the compounds described in the first paper, nor does it seem that good can come from an investigator repeating his own work. The writer sees no more reason to doubt the isomerism of the chlorobromides and bromochlorides of thallium at the present

time than he did a year ago. In conclusion, it may be stated that in so far as it has seemed necessary in this paper to discuss the work of another investigator, there has been no intention of doing more than is demanded by the effort to get at the truth about these interesting and important compounds. If the author has in any way been led into errors or misinterpretations of results, the criticism which is sure to follow will be welcomed with interest. It is recognized that the truth is the important thing, and that a mere matter of opinion is of no value whatever.

ON THE DECOMPOSITION OF HYDROGEN PEROXIDE BY VARIOUS SUBSTANCES AT HIGH TEMPERATURES.

By J. H. Kastle and Mary E. Clarke.

The oxidation of an alkaline solution of chromic oxide to potassium chromate by hydrogen peroxide is one of the well-known reactions of the latter substance. In making a few observations on this oxidation with the view of making chromic oxide the acceptor in the study of certain oxidations by means of atmospheric oxygen, it occurred to one of us (Kastle) to test the conduct of aqueous hydrogen peroxide towards chromic hydroxide in neutral or faintly acid solution. On heating small amounts of the chromic hydroxide with 3 per cent hydrogen peroxide it was observed that oxygen was evolved with an active effervescence when the solution of the peroxide was heated nearly to its boiling-point.

In this connection it occurred to us to test the conduct of a number of other well-known substances towards the peroxide, particularly those, of course, which have no action upon this substance in the cold. As may be seen from the following, certain of these substances catalyzed the peroxide rapidly at 100° C., whereas others were apparently without action upon it at this temperature.

Substances which catalyzed hydrogen peroxide at 100° C.	Substances which do not catalyze hydrogen peroxide at 100° C.
Ferrous sulphate	Sodium chloride
Iron ammonium alum	Sugar
Cupric chloride	Magnesium sulphate[1]
Ferric hydroxide	Zinc oxide
Barium peroxide	Thymol
Copper oxide	Mercuric oxide
Cadmium iodide	Cadmium sulphide
Copper sulphate	Aluminium hydroxide
Potassium dichromate	Oxalic acid
" chromate	
Platinic chloride	
Potassium chlorplatinate	
Cobalt sulphate	
Nickel "	

So far as we have been able to discover in the literature upon this subject, no systematic attempts have ever been made to determine the conduct of substances of different types towards hydrogen peroxide at high temperatures. In his earlier work upon the peroxide, Thénard[2] seems to have reached the conclusion that all substances exert some effect on the peroxide; some, like the acids, to increase its stability; others, like the oxides of metals and finely divided metals, to decompose it.

In 1890 Tammann[3] found that the spontaneous decomposition of hydrogen peroxide in alkaline solution is independent of the nature of the base, and that it is probably brought about by means of certain metallic oxides, such as the oxides of iron, dissolved in the alkali. He observed that the addition of small amounts of such oxides increases the rate of decomposition enormously. Hanriot,[4] Wolffenstein,[5] and others have found that dilute solutions of hydrogen peroxide can be concentrated by heating on the water-bath; in fact, the latter has found that hydrogen peroxide is relatively stable when heated, provided the solution is free from alkaline compounds,

[1] On trying magnesium sulphate more carefully it was found to yield small quantities of oxygen by heating it with the peroxide.

[2] Ann. chim. phys., **9**, 317 (1818).

[3] Ztschr. phys. Chem., **4**, 441.

[4] Compt. rend., **100**, 57, 172.

[5] Ber. d. chem. Ges., **27**, 3307.

and derivatives of the heavy metals, and solids of all kinds, even those that are chemically indifferent.

Quite recently Manchot and Wilhelms,[1] in a communication "On the Peroxide of Iron and the Catalytic Action of Iron Salts," have shown that a solution containing ferrous sulphate and hydrogen peroxide yields considerable quantities of oxygen.

Aside from these few more or less isolated observations, however, we have not been able to find that any study has been made of the action of different substances on hydrogen peroxide at high temperatures. It seemed desirable, therefore, to measure the several amounts of oxygen liberated from hydrogen peroxide at 100° C., or thereabouts, by various substances. This was effected in the following manner: 5 cc. of hydrogen peroxide were placed in a wide-mouth bottle of medium size (175 cc.) and the required amount of the substance, whose action on the peroxide was to be tested, was placed in a small bottle of about 6 cc. capacity, and 5 cc. of water added. This small bottle was then placed in an upright position in the large bottle containing the given quantity of hydrogen peroxide. This large bottle was then tightly stoppered with a single-hole rubber stopper, fitted with a gas-delivery tube, and placed in a bath which was kept filled with boiling water. The gas-delivery tube was then connected with a gas burette. After the level of the water in the gas burette had come to rest, the substance to be tested was brought into the peroxide by slightly tilting and shaking the bottle so as to upset the smaller bottle. The water in the bath was kept actively boiling, and after a certain period the amount of gas in the gas burette was determined. Except when expressly stated to the contrary Marchand's 15-volume solution of hydrogen peroxide, diluted with an equal volume of water, was employed in these experiments. Five cc. of this solution were found to yield 69 cc. of oxygen with lead peroxide and sulphuric acid, temperature 23° C. and 745 mm. pressure.

Five cc. of this solution were used in each experiment, and the substances whose effect on this were to be studied were

[1] Ber. d. chem. Ges., 34, 2485.

employed in the proportion of the thousandth part of their molecular weight in grams, dissolved in 5 cc. of boiling water. The reaction therefore proceeded at a dilution of 10 cc. The results of our experiments, together with all necessary data regarding them, not already mentioned, are given in the following table:

Table I.

Substance used.	Quantity of substance. Gram.	cc. oxygen evolved.	Time.	Temperature. Degrees C.	Pressure.
Water		5.5	15 min.	31	744
Copper sulphate	0.249	44.5	15 "	24.5	725
Cobalt "	0.2811	37.5	15 "	26.5	738.5
" "	"	38	15 "	26	737.5
Nickel "	0.2807	50	15 "	26	"
" "	"	46	15 "	24	739.5
Cobalt ammonium sulphate	0.3951	38.5	15 "	25	"
Cobalt ammonium sulphate	"	40.5	15 "	26	"
Cobalt ammonium sulphate	"	38.5	15 "	26	"
Nickel ammonium sulphate	0.3947	26	15 "	25	"
Nickel ammonium sulphate	"	24.3	15 "	"	"
Ferrous ammonium sulphate	0.392	32.5	15 "	26	"
Ferrous ammonium sulphate	"	32.2	3½ "	"	"
Iron amm'n'm alum	0.964	39.5	15 "	"	746
" " "	"	38.5	10 "	"	"
Potassium alum	0.9484	23	15 "	"	"
" "	"	21.5	15 "	"	"
Potassium chromic alum	0.9983	38	15 "	"	"
Potassium chromic alum	"	38.5	15 "	"	"
Potassium chloride	0.0746	5.5	15 "	27	"
" "	"	7.5	15 "	"	"
" bromide	0.1190	7.5	15 "	"	"
" "	"	7	15 "	"	"
" iodide	0.162	40.5	15 "	26	"
" "	"	38	1 "	"	"

Substance used.	Quantity of substance. Gram.	cc. oxygen evolved.	Time.	Temperature. Degrees C.	Pressure.
Water (alone)	....	8	15 min.	27	745
Zinc sulphate	0.2784	7.5	15 "	"	"
" "	"	9	15 "	"	"
Magn's'm sulphate	0.246	15	15 "	"	"
Ferrous "	0.278	42	15 "	"	"
Manganese "	0.277	30	15 "	"	"
Silver nitrate	0.170	28	15 "	28.5	"
Sodium "	0.085	16	15 "	"	"
Lead peroxide and sulphuric acid	....	56.86	15 "	24	732
Cane sugar	0.342	6.5	15 "	"	"
Sodium chloride	0.0585	7	15 "	"	"
" "	"	8	15 "	"	"
Potassium dichromate	0.2945	46	15 "	26	746
Potassium chromic alum	0.001	20	15 "	27	747
Potassium chromic alum	"	19	15 "	26	"
Cobalt potassium sulphate	0.4373	49.5	15 "	"	"
Cobalt potassium sulphate	"	51	15 "	"	"
Cobalt potassium sulphate	"	54	16 "	27	"
Nickel potassium sulphate	0.4369	25.5	15 "	"	"
Nickel potassium sulphate	"	23.5	15 "	"	"
Benzoyl peroxide	0.242	8	15 "	26	"
Thallous chloride	0.239	18	15 "	27	"

Considerable differences have been observed in the mode of action of a number of these substances. For example, the iron salts, potassium iodide, and chromic alum decompose hydrogen peroxide with great rapidity, whereas, in the case of other substances, such as the nickel and cobalt salts, the rate of decomposition is much slower, although ultimately it is nearly as great. That this is true will be seen from the following table:

Table II.

Substances.	Gram.	Time. Minutes.	Oxygen evolved. cc.
Copper sulphate	0.249	1	37
" "	"	2	41
" "	"	15	44.5
Ferrous ammonium sulphate	0.392	1	31.5
" " "	"	2	32
" " "	"	15	32.5
Iron ammonium alum	0.964	½	39
" " "	"	2	39.5
" " "	"	3	39.5
" " "	"	15	39.5
Potassium iodide	0.162	½	39
" "	"	1	40
" "	"	15	40.5

Among the substances which decompose the peroxide more gradually we have the following:

Potassium chromic alum	0.9983	½	7.5
" " "	"	1	14
" " "	"	2	20
" " "	"	15	38
Nickel sulphate	0.2807	1	6
" "	"	2	14
" "	"	15	46
Cobalt sulphate	0.2811	2	6
" "	"	15	37.5
Cobalt ammonium sulphate	0.3951	1	5
" " "	"	2	11.5
" " "	"	15	38.5
Manganese sulphate	0.277	1	0
" "	"	2	7
" "	"	15	30

The decomposition of the peroxide by certain of these substances, at least, seems to be a true catalysis. This is notably the case with copper sulphate and potassium dichromate, very small amounts of which effect the decomposition of the peroxide. The effect of varying amounts of copper sulphate and potassium dichromate may be seen from the following table:

Table III.

Quantity of copper sulphate used. Gram.	Oxygen evolved. cc.	Time. Minutes.	Temperature. Degrees C.	Pressure.
0.249	44.5	15	24	744.5
0.1245	44	15	25	"
0.0622	44.3	15	28	"
0.0249	44.6	15	27	"
0.0010	44.5	15	27	"
0.0001	38.5	15	28.5	738.5
0.00002	23.1	15	26.5	743
0.00001	18	15	27.5	"

Quantity of potassium dichromate used. Gram.	Oxygen evolved. cc.	Time. Minutes.	Temperature. Degrees C.	Pressure.
0.2945	46	15	26	746
"	45.6	15	27	"
0.0736	45.5	15	26	"
"	44.8	15	26	"
0.001	45.5	15	27	"
"	49	15	26	"
0.00002	15	15	27	"
"	17	15	27	"

When it is borne in mind that all of our experiments were carried out in a total volume of 10 cc., it is evident that 1 part of copper sulphate and 2 parts of potassium dichromate per million are sufficient to effect the decomposition of the peroxide at 100° C.; and about the only difference that could be observed in the action of the copper sulphate and potassium dichromate at different dilutions was that at greater dilutions the decomposition proceeded much more slowly than with large amounts of these compounds. Some idea of the difference in the rate of decomposition may be gathered from the following:

Quantity of copper sulphate. Gram.	Time. Minutes.	Oxygen evolved. cc.
0.249	1	37
"	2	41
"	15	44.5
0.1245	1	19
"	2	23

Quantity of copper sulphate. Gram.	Time Minutes.	Oxygen evolved. cc.
0.1245	15	44
0.0622	1	32
"	2	36
"	15	44.3
0.001	1	21
"	2	34
"	15	44.5
0.0249	1	31
"	2	42
"	15	44.6
With smaller amounts than 0.001 gram :		
0.0001	1	6
"	2	10.5
"	15	38.5
"	23	50.8
0.00002	1	2
"	2	5.5
"	15	23.1
"	20	27
"	23	33
0.00001	1	1.5
"	2	2.5
"	15	18

From the comparatively few cases thus far studied it is impossible to arrive at any general conclusions concerning the cause of these decompositions of hydrogen peroxide, and a great deal more work will have to be done before we can hope to elucidate the matters here under consideration. It is interesting to note, however, in this connection, that of those substances that are capable of causing the decomposition at 100° C. some of them, such as potassium iodide and ferrous sulphate are chemically acted on by the peroxide, and most of those that cause the decomposition of the peroxide contain elements, such as copper and iron, that are capable of combining with oxygen in several different proportions. It may be, therefore, that these several substances capable of bringing about the decomposition of hydrogen peroxide, first combine with it to produce an even more unstable derivative than the hydrogen peroxide itself, and that this then decom-

poses into oxygen, water, and the substance originally causing the catalysis; or looked at from a slightly different point of view, an unstable peroxide may be formed by the action of the hydrogen peroxide on the catalyser, which, by its decomposition, gives oxygen and the original substance. In this connection it is interesting to note that in the decomposition of hydrogen peroxide by finely divided copper a certain interval elapses after bringing these two substances together before the decomposition begins, and during this interval the metallic copper becomes black. This points to the formation of copper peroxide as preliminary to the catalysis of hydrogen peroxide by finely divided copper. A similar explanation has recently been advanced by Manchot and Wilhelm[1] to account for the catalytic action of iron salts on hydrogen peroxide. It would seem probable from these observations that a great many substances will be found capable of decomposing hydrogen peroxide at high temperatures, whereas a great many others will doubtless be found to exert no influence on the decomposition of this compound. To determine just what kinds of substances will and what will not effect this decomposition, and to determine also, if possible, the cause of such decomposition would seem to offer a large and fruitful field for further investigation. It is our intention, therefore, to make a thorough study of these phenomena.

STATE COLLEGE OF KENTUCKY,
LEXINGTON, KY., Oct., 1901.

PHENOLPHTHALIN AS A REAGENT FOR THE OXIDIZING FERMENTS.

BY J. H. KASTLE AND O. M. SHEDD.

In spite of the large amount of work that has been done on the oxidizing ferments since their existence in the living cell was first pointed out by Schönbein[2] in 1856, and later by Traube in 1877,[3] their conduct has been tried towards comparatively few reducing agents. The oxidation of hydroqui-

[1] Ber. d. chem. Ges., 34, 2479.

[2] Schaer: "Die neuere Entwickelung der Schönbeinschen Untersuchungen über Oxydationsfermente." Ztschr. f. Biol., 37, 321 (1899).

[3] Ber. d. chem. Ges., 10, 1895 (1877).

none and certain other phenols and of salicylic, benzoic, and formic aldehydes, the production of guaiacum-blue from guaiacum resin, or better from guaiaconic acid, the synthesis of indophenol and similar dyestuffs, the color reaction with guaiacol, and the oxidation of arsenious to arsenic acid, are the principal changes brought about by the oxydases that have been investigated to any extent. In view of this and of the fact that phenolphthalin is readily oxidized to phenolphthaleïn, a substance readily and beautifully lending itself to colorimetric measurements, it occurred to one of us (Kastle) that this leuco-compound might possibly prove a valuable reagent in the study of the oxidizing ferments.

Phenolphthalin, or dioxytriphenylmethanecarbonic acid,

$$C\begin{cases} C_6H_4OH \\ C_6H_4OH \\ C_6H_4COOH \\ H \end{cases}$$

was first obtained by Baeyer[1] in his investigation of the phthaleïns. The compound is readily prepared by reducing phenolphthaleïn, in alkaline solution with zinc dust. It may thus be obtained in the form of a white crystalline substance. According to Baeyer, phenolphthalin is a strong acid. It is almost insoluble in water, but dissolves readily in alkalies with the formation of readily soluble salts, and on boiling with water it readily dissolves the carbonates of the alkaline earths. In solution it is rapidly oxidized by the more powerful oxidizing agents such as potassium ferricyanide and permanganate, and even on exposure to air it is slowly oxidized to phenolphthaleïn.

On testing the conduct of phenolphthalin towards aqueous liver extracts, which are known to contain at least one oxidase, *viz.*, aldehydase, no certain results could be obtained, and while the results of our experiments are not as yet accepted as absolutely conclusive, they seem to indicate that the oxidation of phenolphthalin is not accelerated by the hepatic ferments.

[1] Ann. Chem. (Liebig), **202**, 80.

The conduct of phenolphthalin towards the potato was next tested. According to Traube, the potato contains an oxidizing ferment. This is shown by the fact that aqueous extracts of the potato give guaiacum-blue directly, without the intervention of hydrogen peroxide or ozonized turpentine. Indeed, the conduct of tincture of guaiacum towards the different parts of the potato tuber is exceedingly interesting. If the potato is scraped and the alcoholic solution of guaiacum applied to the fresh surface just beneath the skin, the characteristic blue color is developed immediately; on the other hand, if the guaiacum is applied to the freshly cut surface of the inner parts of the tuber, the blue color does not show itself at once, but develops slowly on standing exposed to the air. It has also been observed that if a potato is cut in two and guaiacum applied to one surface but not to the other, and both left exposed to the air, the one to which the tincture of guaiacum has been applied slowly develops a blue color, whereas the other does not develop the power to turn guaiacum blue directly as one might suppose, but requires fully as much time to develop the blue color as the freshly cut surface. Aqueous extracts, both of that portion of the tuber immediately beneath the cuticle and of the pulp, turn guaiacum blue at once. It is not the purpose of the present communication, however, to enter into a discussion of the exact significance of these results.[1] As surmised by Traube, however, they certainly seem to point to the presence of an oxidizing ferment in the tuber. It occurred to us, therefore, that an extract of the potato, especially of the epidermal portions of the tuber, might prove a suitable solution on which to test the conduct of phenolphthalin, our mode of procedure being as follows: The outer scrapings of 2 or 3 potatoes, of this season's growth, were macerated with water and the mixture filtered and made up to 25 cc. with water. Clear, yellowish-brown solutions were thus obtained, 5 cc. of which required 0.15 cc. N/20 NaOH in order to give a permanent pink color to phenolphthalein.

[1] These and other results reached in the study of oxidations effected by vegetable and animal tissues will be more fully discussed in a subsequent communication.

Tubes were then prepared containing 5 cc. of this extract together with 1 cc. of a solution of sodium salt of phenolphthalin.[1] These tubes were then allowed to stand at the ordinary temperature for certain intervals, at the end of which 1 cc. of N/20 solution of sodium hydroxide was added. With the potato extracts employed this quantity was ample to insure an excess of alkali. Under these conditions, if any of the phenolphthalin had been oxidized, the characteristic color of phenolphthalein was developed on the addition of the alkali. Further, the intensity or depth of this color was an approximate measure, at least, of the extent of the oxidation in any case. In the following the conditions of the experiments, together with the results obtained, are given in detail.

Three tubes were prepared, the contents of which were as follows:

I. 5 cc. of water and 1 cc. of phenolphthaline solution.

II. 5 cc. extract of potato scrapings boiled and 1 cc. of phenolphthalin.

III. 5 cc. extract of potato scrapings and 1 cc. of phenolphthalin.

These three tubes were allowed to stand for half an hour at room temperature, at the end of which time 1 cc. N/20 sodium hydroxide was added to each, with the following result:

Color after Addition of 1 cc. N/20 Sodium Hydroxide.

I. Very faint pink.

II. Yellowish-brown, with just the faintest tinge of pink.

III. Dark-red.

Precisely similar results were obtained with another extract of potato scrapings, in which case the tubes were allowed to stand at the ordinary temperature for an hour before the addition of the alkali. On adding the N/20 sodium hydroxide the water control in this set of experiments showed a faint pink

[1] The solution of phenolphthalin employed in this investigation was prepared in the following manner: 0.05 gram of phenolphthalin was mixed with just enough N/20 sodium hydroxide to dissolve it. A small quantity of phenolphthalin was then added in order to make certain of neutralizing any slight excess of alkali. The solution was then made up to 10 cc. with water and filtered through a dry filter. A perfectly clear, colorless solution of the sodium salt of phenolphthalin was thus obtained, which was found to be perfectly neutral both to litmus and phenolphthalein.

color, the boiled extract a yellowish-brown with a faint pink tinge, the active, unboiled extract a dark-red.

Similar results were obtained with still a third extract. In this series of experiments the tubes were allowed to stand seventeen hours at room temperature. On adding 1 cc. of N/20 sodium hydroxide, the water control showed a decided pink color, the boiled extract gave a yellowish-pink color, and the active extract a dark-red.

Essentially similar experiments have been tried repeatedly since these first experiments were performed, and in every case active, freshly-prepared extracts of the cuticle of the potato oxidized phenolphthalin.

The Stability of the Oxidase of the Potato in Aqueous Solutions.

As yet no attempt has been made to isolate the oxidase of the potato, and in the extracts employed in this investigation it probably occurs in association with quite a number of substances, among them some very powerful reducing agents. It is therefore not surprising that these aqueous extracts of the potato soon loose their oxidizing power. That such is the case may be gathered from the following experiments bearing upon this point. Two tubes, I. and II., were prepared, I. containing 5 cc. of freshly prepared extract of the potato (cuticle) and 1 cc. of phenolphthalin. The tube was allowed to stand an hour at ordinary temperature, when 1 cc. of N/20 sodium hydroxide was added. A dark-red color was produced. Tube II. was made with the same extract after it had stood at ordinary temperature for an hour. It also contained 5 cc. of the extract and 1 cc. of phenolphthalin. After standing one hour 1 cc. N/20 sodium hydroxide was added. A light-red color was produced, by no means as dark as the color obtained with Tube I. This indicates a falling off in the oxidizing power of the potato extract even in so short a time.

A second extract of the potato cuticle was prepared at 3.20 P.M., August 13th. The extract gave a splendid dark-blue with fresh guaiacum, and when allowed to act on phthalin in the usual manner it gave a clear, decided phenolphthaleïn-red in one-half hour, and dark-red in one hour. At 4.55 P.M.,

the same day, the extract still gave the guaiacum reaction, but much less distinctly than at first; and on testing with phthalin for one-half and one hour it gave only a faint pink color in one-half hour and a much lighter red in one hour than when first tested.

In twenty hours and thirty minutes another extract, which had been preserved from putrefaction by thymol and which, when fresh, was quite active, had lost its power both to oxidize phenolphthalin and to turn guaiacum blue.

On the other hand, an extract which had been preserved with thymol for nine days, still gave a pale-blue color with guaiacum at the end of this time. Unfortunately, not enough of this extract was at hand to test its activity towards phenolphthalin.

These results certainly go to show, that on standing at ordinary temperature the extract of the potato rapidly loses its power to oxidize phenolphthalin and guaiacum.

With the view of determining the solubility of the ferment in other solvents besides water, a quantity of potato scrapings was allowed to dry in the air for twenty-four hours at ordinary temperature. The dry mass was ground to a fine powder with coarse white sand, and an aqueous extract prepared and filtered. The clear filtrate gave a clear blue color with guaiacum, not as deep in tint, however, as that produced with the fresh extracts. Its activity toward phenolphthalin was tested in the usual manner. After an hour's action on the phthalin it showed a decided pink color on the addition of 1 cc. N/20 sodium hydroxide. This color was by no means as deep, however, as that obtained with the fresh extracts in the same length of time. The loss of activity on the part of the tissues, after removal from the tuber is by no means as great, however, as that sustained by the active aqueous extracts in the same length of time.

The Distribution of the Oxidase in the Potato.

As already pointed out above, that part of the potato tuber immediately beneath the skin gives the blue color with guaiacum at once, whereas a freshly cut surface

through the pulp only gives the blue color after standing in contact with guaiacum for some time. On the other hand, aqueous extracts both of the outer scrapings of the potato and of the pulp give the guaiacum-blue at once. This would seem to indicate its universal distribution of the oxidase throughout the tissues of the tuber. With the view of testing the correctness of this conclusion, an aqueous extract of the potato pulp was prepared by thoroughly macerating it with water. Five cc. of this extract required 1.5 cc. N/20 sodium hydroxide to produce a permanent pink color with phenolphthalein. It gave a light-blue color with guaiacum at once. Three tubes were prepared, each containing 5 cc. of the extract and 1 cc. phthalin. The extract in one of these tubes was boiled and cooled to ordinary temperature before adding the phthalin, in order to serve as a control. After twenty minutes, 2.5 cc. N/20 sodium hydroxide were added to one of the active tubes and also to the control. The latter showed no trace of pink color, whereas the active tube showed a decided clear pink. The second tube was allowed to stand twenty minutes longer. Two and a half cc. of N/20 sodium hydroxide were then added. A decided red color was produced, slightly deeper in tint than that obtained with the tube that had stood twenty minutes. There can be no doubt, therefore, that the oxidase is present in the starchy pulp of the potato, as well as in the hypodermal layers. Traces of it have even been found in the harder, denser portions of the skin. This was shown by carefully removing the skin of the potato and washing and scraping thoroughly so as to remove any of the hypodermal tissues. The skin was then allowed to dry in the air for twenty-four hours, when it was ground to a fine powder with coarse white sand. The powder was then extracted with water and the extract filtered. The filtrate gave a very faint-blue with guaiacum. In order to test its activity towards phenolphthalin, a tube was prepared containing 5 cc. of the extract with 1 cc. phthalin. This was allowed to stand one hour. One cc. N/20 sodium hydroxide was then added, when a very light-pink coloration was produced. A control experiment showed no coloration in the same length of time. It is evident, therefore, that even the

skin of the potato contains small amounts of the oxidase.

Effect of Time on the Oxidation of Phenolphthalin by the Oxidase of the Potato.

Even the oxidation of guaiacum by the potato oxidase, while very rapid, is still by no means an instantaneous process, and the oxidation of the phthalin proceeds much more slowly. One advantage of phenolphthalin over a number of substances hitherto proposed as reagents for the oxidases is that the rate of oxidation can be easily and accurately determined by colorimetric comparisons with alkaline solutions of phenolphthaleïn of known strength. While we have not yet had an opportunity to do this, a sufficient number of comparisons has been made to indicate that up to a certain limit, at least, the amount of phenolphthalin oxidized is proportional to the time of its remaining in contact with the oxidase. This may be seen from the following experiments: Tubes were prepared containing 5 cc. of a freshly prepared extract of potato scrapings and 1 cc. phthalin. These were allowed to stand for different intervals, at the end of which 1 cc. N/20 sodium hydroxide was added and the color noted. The following results were obtained:

Time.	Color produced on addition of N/20 NaOH.
1 min.	Scarcely perceptible
5 "	Distinctly pink
15 "	Light-pink
30 "	Decided pink
60 "	Dark-red

It will be seen that the amount of oxidation is proportional to the time. It should be noted in this connection that the oxidation apparently reaches a maximum in one hour. Tubes that have stood for even twenty-four hours give no darker red on the addition of the alkali than those that have stood for an hour. This would indicate either that the oxidase is inhibited in its action by the products of the reaction, or that the oxidizing ferment is really some unstable oxygen compound which by its decomposition oxidizes the reducing agents which are present. The presence of such a compound in the

living cell would account satisfactorily for the oxidation of a limited amount of phenolphthalin.[1]

The Influence of Air on the Oxidation of Phenolphthalin by Potato Oxidase.

In this connection one would naturally be led to inquire whether it is the oxygen of the air which oxidizes the phthalin, the oxidase acting simply in the capacity of an oxygen carrier, or whether the oxygen is supplied by the oxidase itself.

In order to determine, therefore, whether the potato oxidase is an oxygen carrier or merely an unstable oxidizing agent, tubes were prepared, each containing 5 cc. of a freshly prepared potato extract and 1 cc. phthalin. Two tubes were allowed to stand undisturbed in the usual manner for fifteen minutes and one hour, respectively. Through a second pair of tubes a current of air was passed for fifteen minutes and one hour, respectively. The two tubes that had been allowed to run for fifteen minutes showed no difference in color on the addition of 1 cc. N/20 sodium hydroxide; and of the two tubes that had been allowed to run for one hour, the one which had stood quietly during this time gave a somewhat darker tint on the addition of 1 cc. N/20 sodium hydroxide than the tube through which the current of air had passed. From this it would seem that the oxidase of the potato is not an oxygen carrier in the ordinary sense in which this term is employed.[2]

The Occurrence and Distribution of the Potato Oxidase.

This particular oxidase which turns guaiacum blue directly and oxidizes phthalin, is by no means confined to the potato

[1] Further work on this part of the subject is now well under way in this laboratory. The results of the investigation will be embodied in a separate communication by Kastle and Loevenhart.

[2] It should be observed in this connection, however, that in 1848 Schönbein (*Cf.* Schaer: Ztschr. f. Biol., 37, 32 (1899)) showed that air is necessary for the production of guaiacum blue by the potato. This has been confirmed in this laboratory. It would seem, therefore, that while air is necessary for the production of the oxidizing ferment, the latter cannot transfer unlimited amounts of atmospheric oxygen like a true oxygen carrier. The exact significance of this peculiar conduct on the part of the oxidizing ferment of the potato will be discussed in the subsequent communication, to which reference has already been made above.

tuber, but is widely distributed, especially in the vegetable kingdom. As yet, the only animal secretion in which we have found it is human saliva. Without entering into all of the experimental details, there is given in the following table the results of our search for this oxidase in vegetable and animal tissues. In every case aqueous extracts or solutions were employed, and 5 cc. of the extract used in each experiment, together with 1 cc. of the phenolphthalin solution. The acidity of every extract and solution was determined previous to the experiment, in order to know just how much N/20 caustic soda should be added to render the liquid alkaline at the conclusion of the test. It should be observed in this connection that all vegetable extracts used in these experiments had an acid reaction. Hence the oxidation of the phenolphthalin took place in acid solution, and the results in the following table are given for such solutions only. It was observed, however, that the oxidation of phenolphthalin takes place most rapidly in slightly alkaline solutions, and in using this substance as a reagent for the oxidizing ferments it may be well, especially with extracts of weak oxidizing power, to work in alkaline solution. In such solutions, however, it should be borne in mind that the control experiments do not remain colorless even for comparatively short intervals, but always develop a pink color, lighter, of course, in tint than the active extract. This is due to the fact that the oxidation of phenolphthalin by air alone proceeds far more rapidly in alkaline than in acid or neutral solutions. In our experiments the conduct of every extract was also tested towards a fresh alcoholic solution of guaiacum as well as towards phenolphthalin; and all experiments were checked by suitable controls. The results obtained are set forth in the following table:

Table I.

Substance.	Color with guaiacum.	Duration of exp. with phthalin.	Vol. N/20 NaOH added.	Color produced by N/20 NaOH.
1. Ipomoea Batatas: Sweet potato, skin	Dark-blue	40 min.	1 cc.	Dark-red
" " "	"	60 "	1 "	"
2. Zea Mais: Silk of green corn	Blue	30 "	1 "	Pink
" " " "	"	60 "	1 "	Deeper pink than obtained in 30 minutes
3. Musa sapientum: Banana, pulp, ripe	Blue	30 "	2 "	Pink
" " "	"	60 "	2 "	More pink than in 30 minutes
4. Gum arabic	Blue	2 hours	1 "	Decided pink
5. Gum tragacanth	Very faint-blue	2 "	1 "	Very light-pink
6. Cherry tree gum, fresh	Light-blue	2 "	1 "	Decided pink
7. Pine tree gum, fresh	Very faint-blue	4 "	1 "	Slight but decided pink
8. Lycopersicum esculentum Tomato, green fruit	None	17 "	4 "	None
9. Allium Cepa: Onion, bulb	None	7 "	2 "	None

Table I (Continued).

Substance.	Color with guaiacum.	Duration of exp. with phthalin.	Vol. N/20 NaOH added.	Color produced by N/20 NaOH.
10. Malt, infusion, fresh	Light-blue	5 hours	2 cc.	Faint pink
11. Agaricus campestris: Common mushroom	None	4½ "	1 "	None
12. Lepiota Morgani	Dark-blue	1 hour	1 "	Pink
13. Nicotiana Tabacum: Tobacco, Kentucky Burley	Dark-blue	½ "	1 "	Pink
14. Glycinehispida: Soy bean, pod	Dark-blue	1 "	1 "	Dark-red
" " stem	"	15 min.	1 "	"
15. Dioscorea divaricata (Cinnamon vine): Axillary tubers	Dark-blue	½ hour	1 "	Dark-pink
16. Saliva, human	Pale-blue	3 hours	1 "	Decided pink
17. Testicle of sheep	None	20 "	2 "	None

It will be observed that there is a complete parallelism between the oxidation of guaiacum and phthalin by active extracts of living tissues. An extract which gives a deep-blue color with guaiacum will oxidize more phthalin in a given time than one which shows only a faint blue with the former reagent. For example, the potato and sweet potato give a darker blue with guaiacum and oxidize more phthalin in a given time than the silk of the maize or saliva. Further, substances which do not give a blue color direct with guaiacum do not oxidize phenolphthalin at all. Examples of this kind, among plants, are the onion and tomato, and among animal tissues, the liver and testicle of the sheep. In this connection it is interesting to note that those vegetable tissues which readily turn guaiacum blue and oxidize phthalin, readily turn brown or reddish in color when their freshly cut surfaces are exposed to the air; such, for example, are the potato, sweet potato, and banana; whereas, those tissues which do not turn guaiacum blue or oxidize phthalin, such as those of the onion and tomato, do not alter in color when their freshly cut surfaces are exposed to the air. This is in confirmation of the views of Lindet,[1] who believes the darkening of apple juice to be due to an oxidase which oxidizes the tannin of the fruit.

Further, our experiments would seem to indicate that the oxidase concerned in the bluing of guaiacum and the oxidation of phthalin is not identical with the catalase recently described by Loew;[2] for this reason the power of any of these tissues to decompose hydrogen peroxide is not at all proportional to its power to oxidize either guaiacum or phthalin. For example, aqueous extracts of hog liver, sheep testicle, and the common mushroom decompose hydrogen peroxide with the greatest vigor, whereas none of them act upon guaiacum or phthalin; on the other hand, the fresh, green leaf of the tobacco decomposes hydrogen peroxide slowly, whereas it oxidizes guaiacum and phthalin rapidly.[3]

[1] Compt. rend., **120**, 370 (1895).

[2] U. S. Department of Agriculture, Report No. 68.

[3] On the other hand, Spitzer (Pflüg. Arch., **67**, 615 (1897)) is of the opinion that the oxidation of salicylic aldehyde and the decomposition of hydrogen peroxide, by

In conclusion it may be said that, from the results already obtained, it would seem that phenolphthalin will prove a valuable reagent in the study of oxidation processes in animals and plants. It certainly affords a beautiful method for demonstrating the presence of certain oxidases.

STATE COLLEGE OF KENTUCKY,
LEXINGTON, August, 1901.

ON THE NATURE OF CERTAIN OF THE OXIDIZING FERMENTS.

BY J. H. KASTLE AND A. S. LOEVENHART.

In a series of remarkably interesting investigations extending over a period of nearly twenty years (1848–1867), Schönbein[1] demonstrated the occurrence of oxidases (Sauerstofferreger and Sauerstoffträger) in a large number of plants and also in certain animal tissues. Somewhat later, in 1877, Traube[2] introduced the conception of oxidizing ferments. According to Traube these oxidizing ferments are of two kinds, *vitale* and *faulige*, or those operating in higher living organisms and those present in dead matter. Among the first he included the guaiacum-bluing ferment of the potato and the coloring-matter of the red blood corpuscle. Since then the subject of the oxidizing enzymes and the part played by them in oxidations in the living cell has claimed the attention of a large number of observers. Among the oxidations studied in this connection may be mentioned the synthetic oxidation of naphthol and *p*-phenylenediamine to indophenol by Ehrlich; the syntheses of certain indamines and eurhodines by Rohmann and Spitzer; the production of a garnet-red coloring-matter from guaiacol by Bourquelot; the formation of quinone from hydroquinone by laccase, a ferment first obtained by Yoshida, and more completely investigated by Bertrand; and lastly, quantitative measurements of the rate of oxidation of salicylic aldehyde, first by Schmiedeberg and his pupils, and

animal extracts at least, are due to the same cause. On the other hand, E. Lepinots (Compt. rend. de la Soc. de Biol., May 26, 1899) did not observe a parallelism to exist between the quantities of oxygen developed from hydrogen peroxide and the intensity of other reactions, as that upon guaiacum and guaiacol, etc.

[1] See résumé of Schönbein's work by Schaer: Ztschr f. Biol., 37, 321 (1899).

[2] Ber. d. chem. Ges., 10, 1985 (1877).

later by Medwedew and by Jacoby. Besides the work of Schönbein these probably include the more important investigations that have been undertaken in this field.

Except for the work of Bertrand on laccase, Jacoby on salicylase, and Loew on catalase, few if any attempts have been made to isolate the oxidizing ferments, and apart from their destruction by heat and a few poisons, but little is known concerning them which would lead us to regard them as of an enzymic nature. In fact, they do not seem to act as oxygen carriers at all in the sense of being able to transfer large amounts of oxygen from the air to the oxidizable substance. Medwedew observed in his study of the oxidation of salicylic aldehyde to salicylic acid by animal tissues, that this oxidation cannot be increased by an increase in the amount of oxygen present; in other words, this oxidation is independent of the presence of air. He is therefore of the opinion that the oxygen required for such oxidations is derived either from oxyhæmoglobin or from proteid (Pflüger's intramolecular oxygen).

Then, again, it seems never to have been satisfactorily established that the oxidations effected by the so-called oxidases are really catalytic processes in the true sense, *viz.*, that a very small quantity of the substance can, in the presence of free oxygen, effect the oxidation of a relatively large amount of the oxidizable substance. In this connection, however, it should be mentioned that Jacoby[1] has shown in his recent investigation of aldehydase that if the salicylic acid produced is removed by dialysis, the enzyme still possesses some activity. Unless the aldehydase were present in very large quantities in Jacoby's solutions, or these contained unusually large amounts of active intramolecular oxygen, these results of Jacoby are somewhat difficult to reconcile with those obtained by Medwedew, in which he found that atmospheric oxygen was not necessary for the oxidation of the aldehyde.

In attempting to follow the formation of starch in the sprouts and growing tuber of the potato, we had occasion to test different portions of the tuber and sprout with tincture of guaia-

[1] Ztschr. physiol. Chem., 30, 134 (1900).

cum. It is certain that both Schönbein and Traube, and perhaps others before them, were familiar with the fact that the potato causes the bluing of guaiacum. On the other hand, it was observed that different portions of the tuber and sprouts of the potato conduct themselves differently towards the guaiacum. These differences in conduct appeared to warrant further study, as the result of which these observations are submitted.

When the skin of the potato is removed and fresh tincture of guaiacum added to the exposed part, a splendid blue color at once appears. On the other hand, if a potato is cut in two and fresh tincture of guaiacum applied to the freshly cut surface, the blue color develops at once, only in a ring immediately beneath the skin. On the pulp of the potato the blue color does not appear at once, but only on standing. After a time, however, the surface of the pulp turns a dark-blue. Extracts of the skin of the potato and of the pulp both give the guaiacum-blue at once. The former extract, however, gives a much darker blue with the same quantities of guaiacum. It has also been observed that if a few drops of an old tincture of guaiacum be applied to the freshly cut surface of the potato, a faint blue is instantly produced. This, however, rapidly fades out, but after a time returns and increases in amount until a dark-blue coating is produced. The substance producing the blue color with guaiacum is very soluble in water. Scrapings of the skin of the potato need only to be covered with water for a few minutes and the extract filtered, to give a clear yellowish-brown solution, which instantly gives a dark indigo-blue with fresh tinctures of guaiacum. Such solutions, however, rapidly lose strength, and at the same time become darker brown in color. Weak antiseptics, such as chloroform, toluene, and thymol,[1] tend to preserve the power of such extracts to turn guaiacumblue directly; but even solutions preserved in this manner ultimately lose this property. The following data may be cited as showing the stability of such solutions:

A freshly prepared extract of the outer scrapings of the

[1] There is a possibility, of course, that some peroxide may be produced by the slow oxidation of these antiseptics.

potato was found to give a deep-blue color instantly with tincture of guaiacum. Four tubes were prepared as follows:

No. 1 contained the extract alone.

No. 2 contained the extract, together with a small quantity of chloroform.

No. 3 contained some of the extract simply covered, not shaken, with a layer of toluene about 1 centimeter deep.

No. 4 contained the extract together with a small amount of thymol.

These tubes were allowed to stand exposed to the air for twenty-four hours. At the end of this time 1 cc. of the extract from each of the tubes was drawn out and tested with guaiacum with the following results:

No. 1. No blue color.

No. 2. Blue at once (not as deep a blue as the original).

No. 3. No blue.

No. 4. Blue at once, about same tint as No. 2.

These experiments seem to indicate that the oxidizing substance (ferment) of the potato is unstable, and that its stability is increased by certain antiseptics. Its instability is probably due to the deoxidizing action of the reducing substances present in such extracts. As stated above, these extracts become darker brown in color on standing, and this change of color is ordinarily ascribed to oxidation.

It is interesting to note, in this connection, that those extracts of the potato peel which have lost their power to turn guaiacum blue directly, give the guaiacum reaction again on the addition of hydrogen peroxide. As is well known, a number of extracts of vegetable and animal tissues give a blue color with hydrogen peroxide and guaiacum, which do not give the guaiacum reaction alone. The significance of these results will be discussed in a subsequent part of this paper.

The Nature of the Guaiacum Reaction.

Schönbein, Traube, and the earlier workers in this field seem to have entertained no doubt that the formation of guaiacum-blue is a true oxidation. More recently, however, Nasse and Framm[1] have attempted to show that the bluing

[1] Pflüg. Arch., 63, 203 (1896).

of guaiacum tincture does not depend on oxidation by atmospheric oxygen, but must be regarded as a hydrolysis of the guaiacum, since, according to them, this reaction takes place in complete intensity, even when the solutions used are completely freed of oxygen. Hence these observers do not look upon guaiacum as a trustworthy reagent for active oxygen. That oxygen, in some form at least, is necessary for the production of guaiacum-blue seems to have been established by the work of Doebner and Lücker[1] on the guaiacum resin. According to these observers, guaiaconic acid, which composes about 50 per cent of guaiacum resin, yields guaiacum-blue on oxidation, thus:

$$\underset{\text{Guaiaconic acid.}}{C_{20}H_{24}O_5} + 3O \quad = \quad \underset{\text{Guaiacum-blue.}}{C_{20}H_{20}O_6} + 2H_2O.$$

That atmospheric oxygen is essential to the formation of guaiacum-blue by the oxygen carrier (ferment) of the potato has been demonstrated by Schönbein,[2] who showed that the fresh potato in an atmosphere free from oxygen does not give the guaiacum reaction at all. In view of our own ideas upon this subject, and in view, further, of the contradictory statements of Nasse and Framm upon this point which have been referred to above, it seemed to us very important to repeat Schönbein's experiments on this subject. In order, therefore, to determine whether free oxygen is necessary for the guaiacum reaction as brought about by the potato, a potato was placed in a small cylinder, so arranged that a current of hydrogen could gradually replace the atmospheric air which it at first contained. Hydrogen was then passed through the cylinder until all the air had been replaced. The skin of the potato was then scratched by means of a glass rod with sharp edges running through one of the holes of the rubber stopper, closing the cylinder. A drop of fresh guaiacum was then added by means of a separating-funnel without the production of any blue color whatever. The abraided portion of the potato remained white for four hours in the current of hydrogen. The gas was then turned off, the cylinder closed

[1] Pharm. Arch., 234, 590 (1896).
[2] Cf. Schaer: *Loc. cit.*

with paraffin, and all rubber connections were fastened tight by means of screw-cocks. In this condition the cylinder containing the potato was allowed to stand seventeen hours, when the abraided portion showed only the faintest blue color. Just as soon, however, as the potato was removed from the cylinder and exposed to the air a dark-blue color developed on the abraided part. The slight bluing observed undoubtedly resulted from leakage. This experiment was repeated several times with similar results.

In order to make sure that these effects were not the result of any reducing action on the part of the hydrogen, the same experiments were repeated in carbon dioxide and in nitrogen.

Carbon dioxide was passed through the cylinder containing the potato until the escaping gas was completely absorbed by caustic soda. On scratching the surface of the potato with a glass rod and applying guaiacum, no trace of blue color was developed, even after standing for fifteen minutes. On removal to the air, however, a dark-blue color at once developed on the abraided surface. This experiment was repeated several times with the same result in every case. It was found that, if the potato is scratched and allowed to remain exposed to the air for a very short time before putting it into the atmosphere of carbon dioxide, a delicate blue color appears on the scratched surface. This speedily fades away entirely, in the carbon dioxide, leaving the abraided surface perfectly white. This experiment was also repeated with the same result.

Finally, in order to put the matter regarding the influence of air on the guaiacum reaction beyond doubt, the same experiments were tried in an atmosphere of nitrogen. Accordingly, pure nitrogen was passed through the cylinder containing the potato until the air was displaced. The surface of the potato was then scratched and guaiacum applied. No blue color appeared, and the abraided surface remained white indefinitely. On exposure to air, however, the scratched surface at once turned blue. When the potato was scratched in the air and then put at once into an atmosphere of nitrogen and guaiacum applied, the abraided surface developed a faint blue color which rapidly bleached.

A very simple method of showing the influence of atmospheric oxygen on the bluing of guaiacum by the potato consists in making two parallel scratches on a potato, one some little time before applying the guaiacum, the other immediately before its application. On adding the guaiacum simultaneously to both of the scratched surfaces the one which had been made first developed a dark-blue instantly, while on the other surface the blue color showed more faintly and developed more slowly. These trials were made several times, so that there is no doubt that exposure to the atmosphere for a short time before the application of the guaiacum very markedly accelerates and intensifies the formation of guaiacum-blue.

Taken collectively, these results furnish a certain and convincing confirmation of Schönbein's observations, and leave no room for doubt that free molecular oxygen is essential for the change under consideration. It should also be noted, in this connection, that the formation of guaiacum-blue is not the only oxidation that can be effected by the potato. It also gives the garnet-red color with guaiacol, described by Bourquelot, as characteristic of certain of the oxidases. We have also found an aqueous extract of potato scrapings capable of oxidizing salicylic aldehyde, and in this connection Kastle and Shedd[1] have recently shown that active extracts of the potato have the power of oxidizing phenolphthalin to phenolphthaleïn.

The Conduct of Peroxides towards Guaiacum.

Reference has already been made above to the fact that extracts of potato scrapings which have lost their power to turn guaiacum blue directly, regain their power to do this by the addition of very small amounts of hydrogen peroxide, and yet, ordinarily, hydrogen peroxide alone does not give a blue color with guaiacum. Indeed, the power to give guaiacum-blue with hydrogen peroxide and tincture of guaiacum is characteristic of a large number of plant and animal extracts.[2] It

[1] This JOURNAL, **26**, 528.

[2] Schönbein's test for hydrogen peroxide by means of tincture of guaiacum and extract of malt is based upon this fact.

occurred to us, in this connection, to test the conduct of other peroxides towards active animal and plant extracts in the presence of guaiacum. In this work a fresh extract of calve's liver was employed, and it was found that guaiacum-blue was obtained by adding tincture of guaiacum to the liver extract to which a small amount of benzoyl peroxide had been added. Even the boiled liver extract was found to give the guaiacum reaction under these circumstances. It occurred to us, therefore, that the organic peroxides would probably give the guaiacum reaction directly without the intervention of animal or plant extracts at all.

In order to test this point, benzoyl, phthalyl, and succinyl peroxides were prepared according to the well-known methods of von Pechmann and Vanino, and Vanino and Thiele. Small amounts of these peroxides were shaken with small quantities of distilled water and a drop of tincture of guaiacum added, when, without exception, they gave the guaiacum-blue at once and without the addition of any plant or animal extract at all. These results have been obtained repeatedly both with old and perfectly fresh tinctures of guaiacum, so that there can be no doubt about the conduct of these substances in this respect.

In our first experiments with these organic peroxides, no particular pains were taken to get them in solution. In fact, phthalyl and succinyl peroxides are practically insoluble in all common solvents. In testing them with guaiacum, therefore, they were simply mixed with water, and the tincture of guaiacum added at once. Better results, however, can be obtained in the case of benzoyl peroxide by boiling some of this compound with water, filtering hot, and cooling the filtrate. The latter, which always contains benzoyl peroxide partly in solution and partly in suspension, gives a fine blue color on the addition of guaiacum.

The results obtained with the organic peroxides naturally led us to test the conduct of guaiacum towards certain of the inorganic peroxides and oxides. Accordingly the conduct of lead peroxide, manganese peroxide, silver oxide, and freshly

precipitated ferric oxide towards guaiacum was tested by shaking small amounts of these oxides with small quantities of water and adding tincture of guaiacum. Guaiacum blue was obtained at once with the peroxides of lead and manganese, and also with silver oxide, but not with ferric oxide.

These results finally led us to test the conduct of hydrogen peroxide towards tincture of guaiacum. The commercial 3 per cent. solution of hydrogen peroxide does not produce a blue color with guaiacum, either in the cold or on boiling. On heating somewhat more dilute solutions of hydrogen peroxide nearly to boiling and adding guaiacum, a faint blue color was always obtained.

On the Nature of the Oxidizing Ferment of the Potato.

Up to this time the oxidizing ferment of the potato has resisted all attempts to isolate it. This is not surprising when we consider that in all probability it is present in the potato in exceedingly small quantities, and that it is very unstable and remarkably soluble in water. It seems to be insoluble in ethyl and amyl alcohol, at least active extracts of it cannot be prepared by the use of these solvents. It is somewhat soluble in glycerin, but does not seem to be precipitable from either its aqueous or glycerin solutions by alcohol. In view of the fact, however, that both the oxidizing substance of the potato and the organic peroxides give the guaiacum reaction directly, it would naturally suggest itself in this connection that the oxidizing substance in the potato may really be an organic peroxide. It should be observed that, while this supposition is as yet only based upon analogy, it is no idle speculation, but one which best explains the oxidation phenomena exhibited by plants and, in all probability, by animals as well. It should be noted also that Bach,[1] from his studies on the function of peroxides in the phenomenon of slow oxidation, has also reached the conclusion that the so-called oxidizing ferments in the blood are simply readily oxidizable substances having a special aptitude for forming peroxides.

[1] Compt. rend., 124, 951 (1897).

Conduct of the Oxidizing Ferment of the Potato Towards Certain Poisons and Antiseptics.

With the view of comparing the oxidase of the potato with other known enzymes in its conduct towards certain poisons, it occurred to us to try the effect of a number of well-known antiseptics on active extracts of the potato. The following antiseptics were employed in the investigation of this phase of the problem: Silver nitrate, mercuric chloride, hydrocyanic acid, hydroxylamine, sulphurous acid, acid sodium sulphite, sodium thiosulphate, caustic soda, ammonia, phenylhydrazine, and hydrochloric, hydrobromic, nitrotoluenesulphonic, oxalic, and salicylic acids.

Series I.—Two cc. of fresh potato extract and 1 cc. of water or antiseptic solution were used in each experiment. The tubes were allowed to stand two minutes after adding the antiseptic before guaiacum was added. Then 0.1 cc. of fresh tincture of guaiacum was added. The following are the results:

Substance used.	Effect produced by guaiacum.
Water	Dark-blue
Potassium chloride, N/10	"
Salicylic acid, N/10	As blue as the experiment with water at first, then bleached
Silver nitrate, N/10	Dark-blue
Mercuric chloride, N/10	"
Hydrocyanic acid, N/10	No blue color developed, but remained yellow

When 1 cc. of N/10 silver nitrate was mixed with 2 cc. of the active potato extract, and the mixture allowed to stand for fifteen minutes, no blue color was produced on adding guaiacum. On standing for ten minutes, however, a blue color developed which ultimately became as deep in tint as that obtained with the extract alone.

In still a third experiment with silver nitrate, an active potato extract and a 10 per cent silver nitrate solution were mixed in equal volumes. The coagulum produced was fil-

tered off and the clear filtrate treated with guaiacum. A beautiful blue color was obtained.[1]

It will be seen that of the substances tried in this series, only hydrocyanic and salicylic acid inhibited the bluing of the guaiacum by the potato extract.

Series II. With N/10 acids.—Two cc. of fresh potato extract and 1 cc. of water, or the solutions of N/10 acids, were employed in these experiments. The acid was allowed to remain in contact with the potato extract one minute before adding the guaiacum. The following are the results:

Substance.	Effect of guaiacum.
Water	Dark-blue
Nitrotoluenesulphonic acid	Dark-blue at first. Then bleached in the order named
Benzenesulphonic "	
Hydrochloric "	
Hydrobromic "	
Salicylic "	
Oxalic "	

A second series with the same acids, under the same conditions as in the preceding, except that the acids were allowed to remain in contact with the potato extract five minutes before adding the guaiacum, gave the following results:

Substance used.	Effect of guaiacum.
Water	Dark-blue
Nitrotoluenesulphonic acid	No blue color developed
Benzenesulphonic "	
Hydrochloric "	
Hydrobromic "	
Oxalic "	
Salicylic "	Light-blue, soon bleaching out.

Series III. With hydrocyanic acid.—In 1867, Schönbein studied the remarkable effects of hydrocyanic acid on the

[1] Some time after these experiments with silver nitrate and mercuric chloride were first tried, it was found that both of these substances oxidized guaiacum slowly. The fact that these poisons exert practically no effect on the oxidizing substance of the potato, was proved by comparing solutions of potato extract and these salts with their aqueous solutions of the same strength, in their power to produce guaiacum-blue. In every case the solutions containing fresh potato extract gave the guaiacum-blue at once, whereas the aqueous solutions gave it only after long standing.

oxidizing ferments, and showed that very small amounts of this acid serve to inhibit all these processes of oxidation, and in many instances stop them altogether. It seemed of interest to repeat these experiments in this connection, using hydrocyanic acid of known strength.. Two cc. of fresh potato extracts were employed, together with varying amounts of hydrocyanic acid of different strengths. The acid remained in contact with the potato extract one minute before adding the guaiacum. The results are as follows:

Substance used.	Effect of guaiacum.
1.0 cc. of water	Dark-blue
0.9 " " " and 0.1 cc. normal HCN	No blue color developed
0.95 cc. of water and 0.05 cc. normal HCN	Very slight blue color
0.99 cc. of water and 0.01 cc. normal HCN	Very light-blue

In these experiments, an excess of guaiacum failed to intensify the color produced by the 0.1 cc. of guaiacum originally added. Three per cent hydrogen peroxide, however, intensified the blue colors produced in the tubes containing 0.05 and 0.01 cc. of the normal acid.[1] It was further observed that hydrocyanic acid fails to destroy guaiacum-blue when once it is formed. One-tenth of a cubic centimeter of normal acid also failed to prevent the formation of guaiacum-blue when hydrogen peroxide was originally present in the fresh potato extract.

A second series with normal hydrocyanic acid was tried, allowing the acid to remain in contact with the potato extract five minutes before adding the guaiacum, with the following results:

[1] In view of the effect of hydrogen peroxide on extracts whose power to turn guaiacum blue has been destroyed by hydrocyanic acid, the use of hydrogen peroxide as an antidote for hydrocyanic acid poisoning, as recently suggested by Herting, is exceedingly interesting (Ztschr. angew. Chem., 1901, p. 585). If this has not already been tried on the animal, it would seem likely, however, that the production of gas emboli might defeat its purpose.

Substance used.								Effect of guaiacum.
1.0	cc.	water						Dark-blue
0.99	"	"	and	0.01	cc.	normal	HCN	No blue color developed in any of these tubes
0.95	"	"	"	0.05	"	"	"	
0.9	"	"	"	0.1	"	"	"	
0.5	"	"	"	0.5	"	"	"	
0.0	"	"	"	1.0	"	"	"	

With N/100 hydrocyanic acid the following results were obtained after allowing the acid to remain in contact with the potato extract for five minutes:

Quantities of N/100 HCN and water used.								Effect of guaiacum.
1.0	cc.	water						Dark-blue
0.9	"	"	and	0.1	cc.	N/100	HCN	Very light-blue
0.95	"	"	"	0.05	"	"	"	Light-blue
0.99	"	"	"	0.01	"	"	"	"

With even the smallest amount of N/100 acid employed there was a marked retardation in the development of the blue color with guaiacum. This quantity, 9 parts in 10,000,000, very nearly marks the limit of the poisonous effect of the acid on the oxidizing substance in the potato.

Series IV. With hydroxylamine.—Hydroxylamine was the next substance tried. This base was prepared by dissolving a weighed amount of the hydrochloride in the desired amount of water and adding the calculated quantity of caustic soda. In our first series of experiments we used N/10 hydroxylamine, and 2 cc. of potato extract. One-tenth cc. of guaiacum was added immediately after the addition of the hydroxylamine.

Volume of water and N/10 hydroxylamine used.							Effect of guaiacum.
1.0	cc.	water	and	0.0	cc.	hydroxylamine	Dark-blue
0.5	"	"	"	0.5	"	"	No blue color developed
0.9	"	"	"	0.1	"	"	
0.95	"	"	"	0.05	"	"	
0.98	"	"	"	0.02	"	"	Very light-blue

Volume of water and N/100 hydroxylamine used.							Effect of guaiacum.
1.0	cc.	water	and	0.0	cc.	hydroxylamine	Dark-blue
0.5	"	"	"	0.5	"	"	No blue color developed
0.9	"	"	"	0.1	"	"	Very light-blue
0.95	"	"	"	0.05	"	"	

Volume of water and N/1000 hydroxylamine used.						Effect of guaiacum.
1.0	cc.	water and	0.0	cc.	hydroxylamine	Dark-blue
0.5	"	"	0.5	"	"	Trace of blue
0.9	"	"	0.1	"	"	Light-blue
0.95	"	"	0.5	"	"	Light-blue

Series V. With phenylhydrazine (free base).—Preliminary experiments with phenylhydrazine showed that it acted so powerfully that it was decided to employ this substance in N/100 solution in making the tests. Tubes were prepared in the usual manner containing 2 cc. of fresh potato extract. To this quantity of potato extract, water and the solution of phenylhydrazine were added in amounts given below, and 0.1 cc. of tincture of guaiacum was added immediately thereafter. The following results were obtained:

Volume of water and N/100 phenylhydrazine used.						Effect of guaiacum.
1.0	cc.	water and	0.0	cc.	phenylhydrazine	Dark-blue
0.5	"	"	0.5	"	"	No blue color developed
0.9	"	"	0.1	"	"	No blue color developed
0.95	"	"	0.05	"	"	No blue color developed

Even an excess of guaiacum failed to bring out any blue color in any of the tubes containing N/100 phenylhydrazine. An excess of 3 per cent hydrogen peroxide produced blue with 0.1 and 0.05 cc. of N/100 phenylhydrazine, but not with 0.5 cc. of this solution.

Series VI. With N/1000 phenylhydrazine (free base).—The conditions were the same as in the preceding series with N/100 solution.

Volume of water and N/1000 phenylhydrazine used.	Effect of guaiacum.
1.0 cc. of water and 0.0 cc. of N/1000 phenylhydrazine	Dark-blue
0.5 cc. of water and 0.5 cc. of N/1000 phenylhydrazine	No blue color developed
0.9 cc. of water and 0.1 cc. of N/1000 phenylhydrazine	Blue (lighter than with water alone)
0.95 cc. of water and 0.05 cc. of N/1000 phenylhydrazine	Blue (lighter than with water alone)

The tube containing 0.5 cc. N/1000 phenylhydrazine gave no color even with an excess of guaiacum. On adding 3 per cent hydrogen peroxide, however, it became blue. About 0.1 cc. N/1000 phenylhydrazine probably represents the limit of the action of this base on active potato extracts. Under the conditions of the experiment, this corresponds to 3.6 parts per million.[1]

Essentially similar results were obtained with phenylhydrazine hydrochloride. Three and six-tenths parts of this salt per million were found slightly to inhibit the oxidizing power of the fresh potato extract towards guaiacum.

Action of Sodium Thiosulphate on Active Potato Extracts.

The effect of sodium thiosulphate on active potato extracts was tried with the following results. In all of our experiments with this substance 2 cc. of an active potato extract was employed. This was always diluted to 3 cc. with water, or sodium thiosulphate, or both. In the first two series of experiments the guaiacum was added immediately.

Volume of water and N/100 thiosulphate used.	Effect of guaiacum.
1.0 cc. of water and 0.0 cc. of thiosulphate	Dark-blue
0.5 cc. of water and 0.5 cc. of thiosulphate	No blue color produced
0.9 cc. of water and 0.1 cc. of thiosulphate	Very faint-blue color
0.95 cc. of water and 0.05 cc. of thiosulphate	Faint-blue color

[1] On account of the similarity between hydrocyanic acid and phenylhydrazine in their conduct towards the oxidizing substance of the potato, it occurred to us that the latter ought to prove a respiratory poison like the former. It has long been known that phenylhydrazine is a dangerous poison. In order to form a better idea of its action and to determine the lethal dose, its effect was tried on a kitten weighing 515 grams. A solution of phenylhydrazine was prepared containing 0.01324 gram or 0.2 grains per cc. One cc. of this solution was injected into the kitten subcutaneously. In two or three minutes the kitten was prostrated, and from this time on until death, which occurred twenty-six minutes after the injection, respiration was feeble and irregular. The heart continued to beat feebly for three minutes after respiration had ceased. The only noteworthy post-mortem condition was the chocolate color of the blood, due doubtless to the formation of methæmoglobin.

Volume of water and N/1000 thiosulphate used.	Effect of guaiacum.
1.0 cc. of water and 0.0 cc. of thiosulphate	Dark-blue
0.5 cc. of water and 0.5 cc. of thiosulphate	Faint-blue
0.9 cc. of water and 0.1 cc. of thiosulphate	Dark-blue
0.95 cc. of water and 0.05 cc. of thiosulphate	"

Similar experiments were tried with N/1000 sodium thiosulphate, allowing the tubes to stand for five minutes before adding the guaiacum. The results were precisely like those obtained with N/1000 sodium thiosulphate by adding the guaiacum instantly.

It will be seen from these results that sodium thiosulphate reacts almost as powerfully on active extracts of the potato as phenylhydrazine, and yet sodium thiosulphate does not act as a poison towards most enzymes. The fact, therefore, that it acts so injuriously on the oxidizing substance of the potato, while silver nitrate and mercuric chloride do not would seem to indicate that the oxidizing substance of the potato is not a true ferment.

Conduct of Peroxides Toward Antiseptics.

In view of the close analogy existing between certain of the peroxides and the oxidizing substance in the potato, it was thought advisable to test the conduct of these substances toward those antiseptics that were observed to be most injurious to the oxidizing power of the potato extracts.

Benzoyl peroxide was the first of these substances tested in this way, the mode of procedure being as follows:

Small amounts of benzoyl peroxide were placed in test-tubes and the antiseptic to be tested was added in known amount. After standing for a certain time, 0.1 cc. of tincture of guaiacum was added and the color noted. Hydrocyanic acid was the first substance tried. On adding 1 cc. of normal acid to a small amount of benzoyl peroxide and allowing the acid to remain in contact with it for fifteen minutes, and then adding guaiacum, a light-blue color was slowly developed. By com-

paring this with the action of benzoyl peroxide in water on guaiacum, it was observed that a very marked retardation in the bluing of the guaiacum had been effected by the hydrocyanic acid. In the same way it was observed that, while a small amount of benzoyl peroxide in 1 cc. of water speedily developed a deep-blue color with guaiacum, the same amount of peroxide failed to give a blue color with guaiacum when mixed with 1 cc. of N/10 *p*-nitrotoluenesulphonic acid, and allowed to stand three minutes.

This led us to try the action of some of these substances on a solution of benzoyl peroxide. This was obtained by boiling a small solution of benzoyl peroxide with water, filtering, and cooling the filtrate. Under these circumstances a very small amount of the peroxide remains in solution. With guaiacum alone, such solutions give a splendid blue color. In the following experiments 2 cc. of the solution were used with 1 cc. of the antiseptic. The latter was allowed to remain in contact with the peroxide two minutes before adding the guaiacum. The following are the results:

Substances used.	Effect of guaiacum.
N/10 *p*-Nitrotoluenesulphonic acid	No blue color developed
Normal hydrocyanic acid	Slight blue color which soon faded
N/10 Hydrochloric "	Slight blue color which soon faded
N/10 Hydrobromic "	Same results as with N/10 HCl

In a second series of experiments the acids were allowed to act on the solution of benzoyl peroxide for fifteen minutes before adding the guaiacum. Other conditions were the same as in the preceding series. The results are as follows:

Substances used.	Effect of guaiacum.
N/10 *p*-Nitrotoluenesulphonic acid	Very slight blue color at first, soon bleached
Normal hydrocyanic acid	No blue color developed except after long standing, then only faint-blue

Substances used.	Effect of guaiacum.
N/10 Benzenesulphonic acid	Light-blue, soon bleached
N/10 Hydrochloric "	Same as with benzenesulphonic acid
N/10 Oxalic "	Same as with hydrochloric acid only bleached more slowly

Benzoyl Peroxide and Phenylhydrazine.

Tubes were prepared containing 2 cc. of a saturated solution of benzoyl peroxide. With 0.1 cc. of guaiacum, this solution gave a splendid blue color. The action of phenylhydrazine on these portions of benzoyl peroxide was tried at the following dilutions, and in quantities indicated below. The results are as follows :

Substances used.	Effect of guaiacum.
0.9 cc. of water and 0.1 cc. of N/100 phenylhydrazine	Only the faintest blue color
0.95 cc. of water and 0.05 cc. of N/10 phenylhydrazine	Very light-blue
0.99 cc. of water and 0.01 cc. of N/100 phenylhydrazine	Much lighter blue than with 1.0 cc. of water alone
0.9 cc. of water and 0.1 cc. of N/1000 phenylhydrazine 0.95 cc. of water and 0.05 cc. of N/1000 phenylhydrazine 0.99 cc. of water and 0.01 cc. of N/1000 phenylhydrazine	All of these showed a marked falling off in color, in the order named, as compared with that produced in the presence of 1.0 cc. of water alone

It will be observed, therefore, that even 3.6 parts of phenylhydrazine in 10,000,000 inhibit the action of benzoyl peroxide on guaiacum.

Benzoyl Peroxide and Hydroxylamine.

In order to test the effect of hydroxylamine on benzoyl peroxide, tubes were prepared containing 2 cc. of a saturated solution of the latter. A N/10 solution of hydroxylamine was used in quantities indicated in the following. One-tenth cc. guaiacum was added at once after the addition of the hydroxylamine. The following are the results :

Substances used.	Effect of guaiacum.
1.0 cc. of water and 0.0 cc. of hydroxylamine	Dark-blue
0.5 cc. of water and 0.5 cc. of hydroxylamine	No blue color developed
0.9 cc. of water and 0.1 cc. of hydroxylamine	Very pale-blue
0.95 cc. of water and 0.05 cc. of hydroxylamine	Light-blue

Precisely similar results were reached in a second series of experiments, in which the same quantities of hydroxylamine were allowed to act on the peroxide for five minutes before adding the guaiacum.

It has been pointed out above that certain metallic peroxides and oxides produce guaiacum-blue with fresh tinctures of guaiacum. It has also been observed, in this connection, that their action on guaiacum is inhibited by the same antiseptics and poisons that prevent the production of guaiacum-blue by organic peroxides and fresh potato extracts. That this is the case may be seen from the following results:

Effect of Phenylhydrazine on the Production of Guaiacum-Blue by Lead Peroxide.

Tubes were prepared containing small amounts of lead peroxide and 2 cc. of water. To these, small amounts of phenylhydrazine were added. The tubes were then allowed to stand a few moments, when 0.1 cc. of tincture of guaiacum was added to each tube. The following results were obtained:

Substances used.	Effect of guaiacum.
1.0 cc. of water and 0.0 cc. of N/100 phenylhydrazine	Dark-blue
0.5 cc. of water and 0.5 cc. of N/100 phenylhydrazine	No blue color developed
0.9 cc. of water and 0.1 cc. of N/100 phenylhydrazine	" " " "
0.95 cc. of water and 0.05 cc. of N/100 phenylhydrazine	" " " "

It will be observed, therefore, that 18 parts of phenylhydrazine per 1,000,000 is sufficient to prevent the production of guaiacum-blue by means of lead peroxide.

The effect of N/100 phenylhydrazine was tried on a second sample of lead peroxide in exactly the manner described above. With water and guaiacum alone this sample of lead peroxide gave a splendid blue color. With 0.5 cc. N/100 phenylhydrazine no blue color was obtained, even after standing, whereas very faint-blue tints were ultimately obtained by adding guaiacum to the tubes containing 0.1 cc. and 0.05 cc. N/100 phenylhydrazine. These experiments were repeated three times on this sample of peroxide with like result.

The effect of N/100 phenylhydrazine on manganese dioxide and silver oxide is not quite so marked as it is on lead peroxide. The experiments were carried out exactly as with lead peroxide.

With 3 cc. water alone, silver oxide gave a dark-blue color with guaiacum.

With 0.5 cc. N/100 phenylhydrazine and 2.5 cc. of water, it gave no color with guaiacum.

With 0.1 and 0.05 cc. N/100 phenylhydrazine, and 2.9 and 2.95 cc. of water, respectively, silver oxide gave a blue color with guaiacum in each case, although not as dark a tint as that obtained with water alone.

Very small amounts of N/100 phenylhydrazine—0.1 and 0.05 cc. with 2.9 and 2.95 cc. of water, seemed to increase the power of manganese dioxide to oxidize guaiacum. On the other hand, manganese dioxide with 0.5 cc. N/100 phenylhydrazine, and 2.5 cc. of water showed only a pale-greenish tint on standing for some time after the addition of guaiacum.

With N/10 phenylhydrazine, it was found that 0.5, 0.1, and 0.05 cc. of the base absolutely prevented the production of guaiacum-blue by silver oxide and manganese dioxide (native).

Hydrocyanic acid also has the power of preventing the formation of guaiacum-blue by silver oxide and lead peroxide, as will be seen from the following experiments:

(1) With 3 cc. of water and a small amount of lead peroxide, guaiacum gave a bright-blue color.

(2) With 2.5 cc. of water and 0.5 cc. of normal hydrocyanic

acid, and the same amount of lead peroxide as in (1), guaiacum gave no blue color.

(3) Same as (2), but with 2.9 cc. of water and 0.1 cc. of normal hydrocyanic acid, guaiacum gave no blue color.

(4) Same as (2) and (3), but with 2.95 cc. of water and 0.05 cc. of normal hydrocyanic acid, no blue color with guaiacum.

With silver oxide similar experiments were tried. With 3 cc. water alone, silver oxide gave a dark-blue coloration with guaiacum. On the other hand, with 0.5, 0.1, and 0.05 cc. of normal hydrocyanic acid, and enough water in each case to make up to 3 cc., no blue color was obtained on adding 0.1 cc. guaiacum.

With 0.5, 0.1, and 0.05 cc. of N/10 hydrocyanic acid, but with the other conditions the same as in the preceding experiments, silver oxide gave no blue color with guaiacum.

Even N/100 hydrocyanic acid was found greatly to inhibit the guaiacum reaction with silver oxide. With 0.5 cc. N/100 acid and 2.5 cc. water, no blue color was developed even on standing, while with 0.1 and 0.05 cc. of N/100 acid, a light-blue color was developed some little time after adding the guaiacum. Even these showed a retardation in the development of the blue color as compared with water. It will be seen, therefore, that even 45 parts of hydrocyanic acid in 10,000,000 parts of water inhibit the oxidation of guaiacum by silver oxide. On the other hand, N/100 acid seemed to exert no effect on the bluing of guaiacum by lead peroxide. If it exerted any influence at all, it was slightly to accelerate the bluing of the guaiacum by lead peroxide, at least, in the proportion of 0.5 an 0.1 cc. of N/100 acid, with 2.5 and 2.9 cc. of water.

The effect of normal hydrocyanic acid on the power of manganese dioxide to turn guaiacum blue was also tried under conditions identical with those under which its effect on lead peroxide was studied. It was found that even normal hydrocyanic acid or an acid of 2.7 per cent, accelerated the bluing of guaiacum by manganese dioxide, both native and precipitated.

It will doubtless be gathered, from these experiments, that while there are slight differences in their mode of action, there are many fundamental resemblances between the general conduct of the oxidizing ferment of the potato and that of certain of the peroxides, organic and inorganic, towards antiseptics. We have reached the conclusion, therefore, that the oxidizing ferment, which causes the bluing of guaiacum directly, and which oxidizes phenolphthalin, etc., is in all probability nothing more than an organic peroxide,[1] which has been produced at the moment of exposure to the air, from one or more of the readily oxidizable substances contained in the cell. This view regarding oxidation phenomena in the plant is in harmony with modern conceptions concerning autoxidation in general, and at the same time it does away with the necessity for such assumptions as those of Lindet,[2] who, in order to explain why a bruised apple turns brown, although it remains white if intact, found it necessary to assume that the oxidizing ferment and the tannin (the substance oxidized) were situated in different cells, and, therefore, were brought together only by rupture of the tissues. According to the view just advocated, the so-called oxidase (peroxide), is not present at all in the intact cell, but only its forerunner, an autoxidizable substance, which, when it comes in contact with the air, by the tissue being bruised, unites with the oxygen to produce the peroxide, or the so-called oxidizing ferment. This then effects the oxidation of other reducible substances, among them tannin, which very probably are to be found in the same cell with it.

The oxidizing substance of the potato is by no means confined to the tuber of this plant, but seems to be widely distributed in the vegetable kingdom. Schönbein, in 1867, showed that the filtered extracts of a number of plants gave the guaiacum reaction, and since then these observations have been considerably extended by other investigators in this field.

[1] As the result of his studies on "The Function of Peroxides in Phenomena of Slow Oxidation," Bach has reached similar conclusions respecting the oxidizing ferments of the blood. These, according to Bach, are simply readily oxidizable substances with a special aptitude for forming peroxides.—Compt. rend., **124**, 951 (1897).

[2] *Ibid.*, **120**, 370 (1895).

Recently Kastle and Shedd[1] have found the same, or at least a similar oxidizing substance to that in the potato, in at least a dozen different vegetable sources. Up to this time we have not been able to isolate the oxidizing substance in pure condition. It has been found to be very soluble in water, and even on the addition of alcohol only a slight turbidity results on standing, and no filterable product is obtained. Nothing, therefore, is known regarding its composition or nature beyond that already set forth above. From what has already been observed respecting its conduct, it is believed, however, that benzaldehyde may be looked upon as the prototype of the substance in the plant which on exposure to air forms the oxidizing substance, and that in the oxidation and carrying of oxygen by benzaldehyde, we have an exact analogy to the oxidation processes occurring in the plant. Baeyer and Villiger[2] have shown that when benzaldehyde is oxidized by atmospheric oxygen, benzoyl hydrogen peroxide is produced:

$$C_6H_5CHO + O_2 = C_6H_5CO.O.OH.$$

A mixture of benzaldehyde and water, shaken with air, turns guaiacum blue directly.

When benzoyl hydrogen peroxide is left in contact with water it forms benzoic acid and hydrogen peroxide:

$$C_6H_5CO.O.OH + H_2O = C_6H_5COOH + H_2O_2.$$

Or, it may oxidize a second molecule of the benzoic aldehyde so as to produce two molecules of benzoic acid:

$$C_6H_5CO.O.OH + C_6H_5CHO = 2C_6H_5COOH.$$

Or, if another reducing agent, such as indigo, which is more easily oxidized by the benzoyl hydrogen peroxide than benzaldehyde, is at hand, it oxidizes this and forms at the same time one molecule of benzoic acid, thus:

$$C_6H_5CO.O.OH + \text{indigo} = C_6H_5COOH + \text{oxidation product of indigo.}$$

In all of these reactions the point of chief interest is that while benzaldehyde can act as a carrier of oxygen, it can carry

[1] *Loc. cit.*

[2] Ber. d. chem. Ges., **33**, 1569 (1900).

only a limited quantity, that is, one atom per molecule, and is itself oxidized to benzoic acid at the same time. In other words, while a carrier, it is not a true catalytic agent in the sense of being able to react again and again with respect to oxygen. This, of course, is for the reason that the carrier itself is oxidized to a substance which does not combine directly with atmospheric oxygen. The same seems to be true of the substance forming the peroxide in the plant. It readily unites with oxygen to form an unstable oxidizing substance, probably a peroxide, and this readily gives up its oxygen to reducing substances, such as guaiacum, phenolphthalin, tannin, etc. It can oxidize only a limited quantity of these substances, however, even in a current of air,[1] and, on standing, active potato extracts soon lose their power to turn guaiacum blue, doubtless for the reason that the oxidizing substance gives up its oxygen to reducing substances present, such as tannin, etc. That such extracts are not capable of carrying a very large quantity of oxygen is probably to be explained by the fact that the original carrier gives up only half of its oxygen to the reducing substances, and at the same time it is itself oxidized to some product which is incapable of taking up further amounts of oxygen, in just the same way that benzaldehyde is always ultimately oxidized to benzoic acid, by half the oxygen which it originally takes up from the air.

As to the nature of the substance in the plant which produces the oxidizing substance on exposure to oxygen, nothing definite can be said at present. It may possibly be an alde-

[1] See the work of Kastle and Shedd upon this point (*Loc. cit.*). In order to determine whether air would restore the activity of potato extracts that had lost their activity, two tubes were taken containing the same amounts of such an extract. Through one of these a current of air was passed for sixty-five minutes, while the other was left in an open tube during this time. On adding guaiacum to the two extracts it was found that the one through which the current of air had passed gave a little deeper blue color than the one which had stood quietly during the time. In all of our experiments in this direction, and they have been repeated several times, we have never succeeded in restoring to these exhausted extracts anything approaching their original activity, and the fact that there is any increase in activity at all may be explained by the supposition that a small amount of the original oxygen carrier may, on first exposure to the air, escape oxidation. On exposing mixtures of benzaldehyde and water to the air, for example, not all of the benzoic aldehyde is oxidized at once but only gradually.

hyde. In fact Curtius and Reinke[1] have obtained from the leaves of certain green plants, by distillation in a current of steam, a di- or tetrahydro aromatic aldehyde-alcohol, to which they assigned the formula

$$C_6H_6\begin{cases}CHO\\CH_2OH\end{cases} \quad \text{or} \quad C_6H_8\begin{cases}CHO\\CH_2OH\end{cases}.$$

Such a substance would probably conduct itself towards guaiacum, etc., in much the same manner as an active potato extract. It is interesting to note, in this connection, that those antiseptics which inhibit the action of potato extracts on guaiacum to the greatest extent are those which most readily combine with aldehydes. On the other hand, it should also be noted that these same substances have proved to be the most powerful in preventing the action of peroxides upon guaiacum. Hence it may be that their power to prevent the production of guaiacum-blue may be due partly to their reducing action and partly to their power to combine with aldehydes.

The Rôle of Oxygen Carriers in the Normal Oxidation Process in the Plant.

It will be observed that we obtain evidence of these oxidizing substances in the plant only by the destruction and rupture of plant tissues, and their free exposure to air. It is pertinent, therefore, to inquire whether these carriers are concerned in the normal oxidation phenomena of the living cell. There is certainly nothing known that indicates that they are not thus concerned, nor is there anything to show that the carrier itself is produced by rupture of the cell and exposure to the air. It would seem, rather, to be a constant product of metabolic activity, and the fact that it so readily combines with oxygen on exposure to air, seems to indicate that it would also combine directly with any oxygen entering the living cell, and thereby participate in the respiratory activities of the plant. The fact that such an oxygen carrier may be present in the cell and yet not render the vegetable tissue brown in color, as always occurs when such tissue is bruised

[1] Ber. d. bot. Ges., 15, 201, 10, 26/3.

and freely exposed to the air, is capable of several explanations. It may be, for example, that with the amount of oxygen normally supplied to the tissue only such small amounts of peroxide are produced that the amount of tannin, and other substances oxidized, is imperceptible so far as their color is concerned; or it may also be that with small amounts of the peroxide present in the tissue at any one time, other more powerful reducing agents appropriate the oxygen of the peroxide as fast as it is formed, and that the tannins therefore escape oxidation altogether. It is also to be observed, in this connection, that the accumulation of the oxygen carrier in the cell, or in any particular tissue, does not necessarily indicate an excessive oxidation for that particular cell or part. Indeed it would seem to indicate the reverse, for in the tissues of warm-blooded animals we do not find any substance capable of turning guaiacum blue directly, whereas in the potato tuber we do. Yet the failure on the part of animal tissues to give guaiacum-blue directly may not be due to the absence of the peroxide substance, but to the presence of substances more readily oxidized by the peroxide than guaiacum.

The Hydrogen Peroxide-Guaiacum Reaction.

There is still another reaction which presents a close similarity to the one under consideration, and about which it seems necessary to say a few words in this connection. That is the reaction which results in the formation of guaiacum-blue directly from tincture of guaiacum and hydrogen peroxide by animal and plant extracts, and which modern biochemists have ascribed to the enzyme called peroxidase. The power of producing guaiacum blue from a mixture of hydrogen peroxide and tincture of guaiacum is certainly a very general property of active animal and plant tissues, much more so, indeed, than the power of these tissues to give guaiacum-blue without the addition of hydrogen peroxide. It has been observed in this connection, for example, that potato extracts which had lost their power to turn guaiacum-blue directly still gave guaiacum-blue on the addition of hydrogen peroxide, either before or after the addition of tincture of guaiacum; and in the same

way a good many animal and plant tissues and secretions which do not give the guaiacum reaction directly, such as blood, liver, etc., readily give it on the addition of hydrogen peroxide. In spite of its wide distribution, however, it is not universally characteristic of living tissues. For example, the onion bulb not only does not give the guaiacum reaction directly, but it does not give it on the addition of hydrogen peroxide. It should be borne in mind, however, as Hunger has recently shown,[1] that this reaction with guaiacum and hydrogen peroxide is sometimes masked by the presence of glucose and other powerful reducing substances in the plant. The fact that hydrogen peroxide does not give the guaiacum reaction directly, or at least but faintly when heated, whereas other peroxides, especially the organic peroxides, do so, would seem to indicate that the function of the hydrogen peroxide in the guaiacum-hydrogen peroxide reaction, is to react with some one or more of the organic substances present in the plant or animal extract to form an organic peroxide. A reaction of this character has been described by Baeyer and Villiger, who have shown that acetic peroxide, one of the most unstable of these derivatives, can be readily prepared by the action of hydrogen peroxide on acetic anhydride. In fact it has been observed in this connection that hydrogen peroxide may react even with the organic acids to form organic peroxides. At least, certain of these, such as benzoic and succinic acids, have been found to give the guaiacum reaction with hydrogen peroxide.

In view of all that has been set forth above, it is believed that the action of hydrogen peroxide in the guaiacum-hydrogen peroxide reaction is much the same as that of free oxygen in the reaction in which the guaiacum-blue is produced directly by the action of the plant extract. We have seen that the latter process can be explained by the formation of an organic peroxide from the oxygen of the air and some readily autoxidizable substance contained in the extract. It would seem probable, therefore, that the action of the hydrogen peroxide on the extract is to convert some organic compound,

[1] Ber. d. bot. Ges., 19, 374.

which would not be acted on in this way by molecular oxygen, into a peroxide.

On the other hand, it is possible that the peroxidase may react in such a manner as to weaken the bonds of the oxygen in the hydrogen peroxide, somewhat after the manner of the catalase described by Loew[1] and others, whereby it becomes able to oxidize the guaiacum. The fact that hydrogen peroxide turns guaiacum faintly blue when heated, and that its powers of oxidation are generally greatly increased by oxygen carriers such as ferrous and ferric salts, would seem to indicate the possibility of such an action on the part of the animal or plant extract. It is our intention to investigate the power of such extracts to act as oxygen carriers in the presence of hydrogen peroxide, particularly in such reactions as the oxidation of formaldehyde and phenolphthalin by hydrogen peroxide. In fact, studies with this end in view have already been begun in this laboratory.

In conclusion, it may be said that the results reached in the present investigation seem to warrant the following conclusions:

1. That oxygen is absolutely essential to the production of the guaiacum-bluing ferment of the potato.

2. That this so-called oxidizing ferment is in all probability not a true soluble ferment, but an organic peroxide.

3. That the oxidation phenomena occurring in the plant, and probably in the animal organism also, can be satisfactorily explained upon the supposition that the readily autoxidizable substances which they contain are oxidized to the peroxide condition by molecular oxygen, and that the peroxides thus formed in turn give up part of their oxygen to other less oxidizable substances present in the cell. In other words, that the process of rendering oxygen active, by the living cell, is probably brought about in essentially the same way that this is accomplished by phosphorus, benzaldehyde and other oxygen carriers, *viz.*, as one phase of autoxidation.

STATE COLLEGE OF KENTUCKY,
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[1] U. S. Dept. of Agr., Report 68.